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Poster

196. Cell Lineage Analysis

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 196.01/A1

Topic: A.01. Neurogenesis and Gliogenesis

Support: RGC Areas of Excellence (AoE/P-705/16)

Title: Caveolin-1 promotes neural-lineage commitment by regulating mitochondrial functions in adult hippocampal neural stem cells

Authors: *G. CHONG, M. WU, Q. DU, R. DENG, J. SHEN;
Sch. of Chinese Med., The Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract: Emerging evidence suggests the importance of mitochondrial functions in regulating early lineage commitment of neural stem cells (NSCs) in adulthood. While, selecting molecules in mediating mitochondrial functions in NSCs would offer the applicable targets for neural regenerative therapy. By observing with super-resolution microscope (dSTORM), we identified Caveolin-1 (Cav-1), the main component of the caveolae plasma membranes, also located around mitochondria in NSCs. It indicates a possible regulatory functions of Cav-1 in mitochondria. Indeed, Cav-1 knockout (KO) mice established the decreased LDHA and Ace-MnSOD level in mitochondrial fragments, suggesting the hyperactivity mitochondrial metabolism induced by deletion of Cav-1. Interestingly, we discovered an increased population of neuroblasts in Cav-1 KO hippocampus without the significant change in cell proliferation by immunofluorescence. Additionally, in Cav-1 KO hippocampus, neuronal production was also elevated but the survive rate of new born neurons was decreased. Hence, Cav-1 depletion may cause an over-activity of mitochondrial metabolism and production of ROS, which induced the increased neural lineage commitment but lower survival rate of the adult generated neurons. This phenomena provide the evidence that Cav-1 would be lineage regulator during process of adult neurogenesis and may be the molecular target for neural regenerative therapy via regulating mitochondrial functions.
Acknowledgement:
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Disclosures: G. Chong: None. **M. Wu:** None. **Q. Du:** None. **R. Deng:** None. **J. Shen:** None.

Poster

196. Cell Lineage Analysis

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 196.02/A2

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH R01 NS095908
NIH T32 NS062443
NIH T32 MH020068
NIH R25 NS080686
Brain Research Foundation BRFSG-2016-11
Agency for Science, Technology and Research of Singapore

Title: Diverse spinal commissural neuron populations revealed by fate mapping and molecular profiling using a novel Robo3^{Cre} mouse

Authors: *A. TULLOCH[1], S. TEO[2], B. V. CARVAJAL[1], M. T. TESSIER-LAVIGNE[3], A. JAWORSKI[1];
[1]Neurosci., Brown Univ., Providence, RI; [2]Rockefeller Univ., New York, NY; [3]President, Stanford Univ., Stanford, CA

Abstract: The two sides of the nervous system coordinate and integrate information via commissural neurons, which project axons across the midline. Commissural neurons in the spinal cord are a highly heterogeneous population of cells with respect to their birthplace, final cell body position, axonal trajectory, and neurotransmitter phenotype. Although commissural axon guidance during development has been studied in great detail, neither the developmental origins nor the mature phenotypes of commissural neurons have been characterized comprehensively, largely due to lack of selective genetic access to these neurons. Here, we generated mice expressing Cre recombinase from the *Robo3* locus specifically in commissural neurons. We used *Robo3^{Cre}* mice to characterize the transcriptome and various origins of developing commissural neurons, revealing new details about their extensive heterogeneity in molecular makeup and developmental lineage. Further, we followed the fate of commissural neurons into adulthood, thereby elucidating their settling positions and molecular diversity and providing evidence for possible functions in various spinal cord circuits. Our studies establish an important genetic entry point for further analyses of commissural neuron development, connectivity, and function.

Disclosures: A. Tulloch: None. **S. Teo:** None. **B.V. Carvajal:** None. **M.T. Tessier-Lavigne:** None. **A. Jaworski:** None.

Poster

196. Cell Lineage Analysis

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 196.03/A3

Topic: A.01. Neurogenesis and Gliogenesis

Title: Hopx in precursors from embryonic ventricular zone instructs generation of late-born cells

Authors: *Q. XIAO;
Inst. of Neurosci., Shanghai, China

Abstract: In recent years, the outer subventricular zone populated with outer radial glial cells(oRGs) in the developing human neocortex attracted a wide spread attention. Among the specific genes expressed by oRGs, Hopx is frequently regarded as a trustable oRG marker in recent studies on human neocortex and cultured organoid. Intriguing, Hopx is a gene not humanoid specific but having prevalent expression at ventricular zone and cortical hem of rodent cortex, which conflicts with the fact that oRGs exist very sparsely in mouse neocortex. Thus, the role of Hopx expressed neural progenitors (NPs) in the developing brain cortex needs to be further illustrated. By using immnunostaining, we visualize that Hopx preferentially expressed in the BLBP-postive NPs in mouse cerebral cortex except of that in the dentate gyrus (DG). Further observation of the postnatal Hopx-CreER mice administrated with tamoxifen prenatally, the fact that Hopx positive precursors are inability to generate deep layer neurons thoughout all stages of cerebral development is identified. Moreover, promotion of neuronal differentiation is found both in the animals with transient knockdown of Hopx and animals with germline-null alleles. In addition, lineage-tracing experiments provide similar evidence in the mouse brain with Hopx area patterning. Collectively, these results show that Hopx instructs generation of late-born cells by repressing of neurogenesis naturally in fate specification in the cerebral development process.

Disclosures: Q. Xiao: None.

Poster

196. Cell Lineage Analysis

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 196.04/A4

Topic: A.01. Neurogenesis and Gliogenesis

Support: KAKENHI 16K07011

NIG-JOINT 33A2018

Title: Genetic labeling of neurons derived from direct and indirect neurogenesis in the mouse cerebral cortex

Authors: *Y. HATANAKA[1], Y. KAWAGUCHI[2], T. HIRATA[3];
[1]Osaka Univ., Suita, Japan; [2]Natl. Inst. Physiol Sci., Okazaki, Japan; [3]Nat'l Inst. Genet., Mishima 411-8540, Japan

Abstract: Diverse types of cortical excitatory neurons are generated from radial glial cells (RGCs). RGCs initially undergo symmetric cell division to amplify progenitors. At the neurogenic stage, RGCs undergo asymmetric cell division to produce progenitors and neurons (direct neurogenesis) or progenitors and intermediate neuronal progenitors (INPs), which further divide to produce neurons (indirect neurogenesis). These processes have been revealed by in vitro imaging techniques. However, it remains obscure if these differentiation modes contribute to generating diverse types of cortical neurons. In this study, we developed a genetic labeling method, which distinguishes neurons from direct and indirect neurogenesis, and a genetic method to label daughter neurons derived from INPs. We used a mouse line (G2A) that expresses CreER^{T2} under the control of a putative neurogenin (Ngn) 2 promoter, because stable Ngn2 expression occurs in cells committed to neurons. Indeed, immunohistochemical study showed that CreER^{T2} expression was basically overlapped with endogenous Ngn2 expression, although slightly shifted to basally and partly overlapped with Tbr2. We crossed the G2A driver line with Ai14 mice (ROSA26-lsl-tdTomato), and administered a limited amount of tamoxifen to G2A:Ai14 pregnant mice. Since INPs retain mitotic activity, combination with EdU administration distinguished neurons generated by direct neurogenesis (tdTomato⁺/EdU⁻) from those generated by indirect neurogenesis (tdTomato⁺/EdU⁺). We also crossed the G2A line with Mosaic Analysis with Double Markers (MADMs) line for labeling mitotic INPs. Tamoxifen administration to pregnant G2A:MADM-11^{GT/TG} mice successfully labeled clonally related neurons from INPs, which were revealed by GFP and tdTomato expression. The present methods enabled us to investigate laminar distributions, molecular phenotypes, and morphological aspects of cortical neurons generated by the two distinct manners, which would help us understand the mechanisms producing various excitatory neurons in the developing cortex.

Disclosures: Y. Hatanaka: None. **Y. Kawaguchi:** None. **T. Hirata:** None.

Poster

196. Cell Lineage Analysis

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 196.05/A5

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant R01 MH100914
NIH Grant U01 MH106876

Title: Somatic mutations reveal left versus right asymmetry during early human development

Authors: *L. FASCHING[1,2], S. TOMASI[1,2], T. BAE[6], M. BRADY[1,2], S. ABDALLAH[1], L. TOMASINI[1,2], N. VASMATZIS[6], A. SZEKELY[3], T. V. FERNANDEZ[1,4], J. F. LECKMAN[1,4], A. ABYZOV[6], F. M. VACCARINO[1,2,5];
[1]Child Study Center, Yale Sch. of Med., [2]Dept. of Neuroscience, Yale Sch. of Med., [3]Dept. of Neurology, Yale Univ. Sch. of Med., [4]Dept. of Psychiatry, Yale Sch. of Med., [5]Kavli Inst. for Neuroscience, Yale Sch. of Med., Yale Univ., New Haven, CT; [6]Dept. of Hlth. Sci. Research, Ctr. for Individualized Med., Mayo Clin., Rochester, MN

Abstract: Somatic mutations develop during embryogenesis and continue to occur over a lifetime. When a somatic mutation arises in a progenitor cell, all daughter cells will share that particular mutation. Therefore somatic mutations can be germ layer, organ, or cell specific and enable tracing cell linages.
Here we describe a 28 year-old male with severe Tourette syndrome (TS) who has been evaluated and followed by our group at the Yale Child Study Center since 1999. His abnormal sensory urges and self-injurious motor tics are manifested exclusively on the left side of his body resulting in several traumatic injuries on the left eye, mouth, and face.
We isolated fibroblasts by skin biopsy from symmetric inner regions of his right and left upper arms, expanded the cells *in vitro* and performed whole exome capture sequencing. Computational analysis predicted 46 and 25 single nucleotide variants (SNVs) present exclusively on the right and the left side, respectively. Of these, 17 right and 13 left were somatic SNVs and validated by amplicon-seq in the original fibroblasts and were carried together in single iPSC lines, suggesting that fibroblasts are clonally distributed within the human dermis. Interestingly, *HTR1A*, *CACNB4*, *NRXN1* and *DBH*, four genes that are associated with brain function, carried missense SNVs in fibroblasts on the right side, contralateral to his symptoms. Virtually all of these SNVs were also detected in the patient's saliva or urine, suggesting that his left and right cell body lineages diverged before the separation of the 3 germ layers at gastrulation. Furthermore, such an early origin implies that these lateralized somatic mutations may be present on the right and left sides of this patient's brain and may be responsible for his asymmetric symptoms. Surprisingly, none of the mutations were detected in blood, suggesting limited use of blood to detect mosaic variants present across the body. Similar analyses in other individuals suggest comparable abundance of lateralized SNVs. We suggest that left-right asymmetry originating before organogenesis is commonplace in humans and may be important for human body and brain laterality and disease.

Disclosures: L. Fasching: None. **S. Tomasi:** None. **T. Bae:** None. **M. Brady:** None. **S. Abdallah:** None. **L. Tomasini:** None. **N. Vasmatzis:** None. **A. Szekely:** None. **T.V. Fernandez:** None. **J.F. Leckman:** None. **A. Abyzov:** None. **F.M. Vaccarino:** None.

Poster

196. Cell Lineage Analysis

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 196.06/A6

Topic: A.01. Neurogenesis and Gliogenesis

Support: NICHD Division of Intramural Research Program

Title: Spatial heterogeneity of mouse telencephalic progenitors at single-cell resolution

Authors: *D. LEE, C. RHODES, Y. ZHANG, T. PETROS;
Unit on Cell. and Mol. Neurodevelopment, NIH/NICHD, Bethesda, MD

Abstract: The cellular complexity of mouse cerebral cortex has been intensively studied, yet a thorough characterization of distinct neurogenic regions of the developing telencephalon at single-cell resolution has not been described. Neurogenesis occurs within the embryonic ventricular zone (VZ) and subventricular zone (SVZ), and a detailed description of progenitors in these niches may give insight into gene pathways regulating brain patterning and initial cell fate decisions. Here, we use single-cell transcriptomics to study heterogeneity within VZ and SVZ in the dorsal telencephalon, source of glutamatergic projection neurons, and the three ganglionic eminences (GEs) that generate distinct populations of inhibitory GABAergic neurons. By harvesting Nestin-expressing cells, VZ progenitors were isolated from all four brain regions and compared to SVZ progenitors and postmitotic mantle zone cells. VZ progenitors show restricted spatio-temporal transcriptional patterns specific to their regions which subsequently lead to differentiation of heterogeneous secondary SVZ progenitors. Furthermore, early cellular response genes were differentially regulated between regions suggesting that highly dynamic morphogenic cues may be responsible for heterogeneity within the neurogenic niches and regions during development. Studies to investigate candidate genes that may regulate intra- or inter-regional cell fate specificity are underway. Our findings characterize the systematic landscape of transcriptional diversification in the developing telencephalon with the hope to uncover fate-determining genes that bring cellular diversification to multipotent telencephalic progenitors.

Disclosures: D. Lee: None. **C. Rhodes:** None. **T. Petros:** None. **Y. Zhang:** None.

Poster

196. Cell Lineage Analysis

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 196.07/A7

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant EY013760
Research to Prevent Blindness, Inc.
NIH Grant P30EY008126

Title: Resolving the role of Lhx2 in the neurogenic output of retinal progenitor cells using single-cell RNA sequencing

Authors: *A. PATEL, A. FULLER, E. M. LEVINE;
Ophthalmology and Visual Sci., Vanderbilt Univ. Med. Ctr., Nashville, TN

Abstract: Introduction: Retinal progenitor cells (RPCs) pass through stages of developmental competence where they successively acquire and lose the ability to generate the different retinal cell classes. Lhx2, a LIM homeodomain transcription factor, is expressed in RPCs throughout neurogenesis and regulates many of their stage-specific properties. We previously showed by immunohistology that Lhx2 inactivation at the start of retinal neurogenesis causes a reduction in RPCs, imbalanced neurogenic output and impaired competence progression. This was evidenced by early cell type overproduction that persisted past the normal period (retinal ganglion cells; RGCs), and a reduction in the generation of rods photoreceptors, a late generated cell type.
Purpose: To more rigorously assess whether the ectopically generated RGCs exhibited lineage fidelity in the absence of Lhx2 and that the early competence state was extended at the expense of the late competence state, we employed single cell RNA sequencing to examine the gene expression profile of individual cells after temporally-controlled Lhx2 inactivation in RPCs.
Methods: Lhx2 was inactivated in RPCs with tamoxifen-dependent Cre recombination at the start of retinal neurogenesis (~E11.5) and samples were collected at E18.5. Control and CKO retinas were dissociated into single cells and barcoded libraries were constructed with the 10X Chromium Controller. Sequencing was done on an Illumina NovaSeq6000. Analysis of single cell transcriptomes were conducted using the R and Python software environments and included the Seurat, CellRanger, pCreode, and scUnifrac packages.
Results: Cluster and nodal analysis revealed differences in the distribution of cell types within the control and cKO samples. Trajectory analysis indicates that RGC production is continuing past their normal period and that by and large, cell identities are maintained with some exceptions. Additionally, there is evidence of differences in the distribution of various cell-types not observed previously.
Conclusions: We were successful in identifying differences in cell fate and differentiation due to

the removal of Lhx2 function. Although analysis is ongoing, our data thus far points to Lhx2 having a key role in allowing RPCs to advance in their competence state from early to late and that by and large, lineage fidelity is maintained in most retinal neurons.

Disclosures: A. Patel: None. **A. Fuller:** None. **E.M. Levine:** None.

Poster

196. Cell Lineage Analysis

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 196.08/A8

Topic: A.01. Neurogenesis and Gliogenesis

Support: NSF Award 1553764
M. J. Murdock Charitable Trust

Title: Multicolor lineage tracing using *in vivo* time-lapse imaging reveals coordinated death of clonally related cells in the developing vertebrate brain

Authors: N. L. BROCKWAY[1], **T. LICHTENBERG**[1], N. HO[1], Z. COOK[1], Y. A. PAN[2], *T. A. WEISSMAN[1];
[1]Lewis & Clark Col., Portland, OR; [2]Virginia Tech., Roanoke, VA

Abstract: The global mechanisms that regulate and potentially coordinate cell proliferation and death in developing neural regions are not well understood. In particular, it is not clear how or whether clonal relationships between neural progenitor cells and their progeny influence the growing brain. We have developed an approach using Brainbow in the developing zebrafish to visualize and follow multiple clones of related cells *in vivo* over time. This allows for clear visualization of many dividing clones of cells, deep in proliferating brain regions. As expected, in addition to undergoing interkinetic nuclear migration and cell division, cells also periodically undergo apoptosis. Interestingly, cell death occurs in a non-random manner: clonally related cells are more likely to die in a progressive fashion than cells from different clones. Multiple members of an individual clone die while neighboring clones appear healthy and continue to divide. Our results suggest that clonal relationships can influence cellular fitness and survival in the developing nervous system, perhaps through a competitive mechanism whereby clones of cells are competing with other clones. We are now testing whether specific factors influence this process, including overexpression of myc and manipulation of the BMP signalling pathway. Clonal cell competition may help regulate neuronal proliferation in the vertebrate brain.

Disclosures: N.L. Brockway: None. **T. Lichtenberg:** None. **N. Ho:** None. **Z. Cook:** None. **Y.A. Pan:** None. **T.A. Weissman:** None.

Poster

196. Cell Lineage Analysis

Location: Hall A

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Topic: A.01. Neurogenesis and Gliogenesis

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University of Connecticut Institute for Brain and Cognitive Sciences (P.J.B., A.A.K)
University of Connecticut Office of Undergraduate Research Supply Award (P.J.B., A.A.K., D.P.)

Title: Stem cell fate decisions in the V-SVZ during normal embryonic and postnatal development

Authors: *S. E. MAY[1], T. NUHAT SHAFIN[2], P. J. BRIODY[2], A. A. KALARIA[2], D. PAN[2], J. C. CONOVER[3];
[1]Physiol. and Neurobio., [2]Univ. of Connecticut, Storrs, CT; [3]Dept Physiol & Neurobiol, Univ. Connecticut, Storrs Manfld, CT

Abstract: The ventricular-subventricular zone (V-SVZ), a stem cell niche located along the walls of the lateral ventricle, generates neurons and glia to populate regions of the forebrain. The V-SVZ also generates an epithelial monolayer of ependymal cells to line the ventricle surface. Along the lateral wall of the lateral ventricles a unique organization of ependymal cells and remaining stem cell processes form regenerative units referred to as ‘pinwheels’ in contrast to the medial wall of the lateral ventricle, which is covered entirely by ependymal cells. We examined the mechanism of stem cell division (symmetric versus asymmetric) that gives rise to new ependymal cells with the retention of clustered populations of stem cells (pinwheels). Using piggyBac *in utero* electroporation together with BrdU/EdU double-labeling of V-SVZ stem cells, we monitored the fate decisions of V-SVZ stem cells during the period of ependymogenesis throughout development in male and female mice. Here, we show the progression of ependymogenesis from caudal to rostral along the ventricle surface of postnatal mice, track the generation of pinwheel units, and monitor stem cell division during different stages of early development.

Disclosures: S.E. May: None. **T. Nuhat Shafin:** None. **P.J. Briody:** None. **A.A. Kalaria:** None. **D. Pan:** None. **J.C. Conover:** None.

Poster

196. Cell Lineage Analysis

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 196.10/A10

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Training Grant 5T32GM007601-39
Brain Research Foundation Seed Grant BRFSG-2016-11

Title: Elucidating transcriptional trajectories of spinal commissural neuron subtypes

Authors: *J. ABOLAFIA[1], A. J. TULLOCH[2], D. CROOTE[2], A. JAWORSKI[2];
[1]Mol. Biology, Cell Biology, and Biochem., [2]Neurosci., Brown Univ., Providence, RI

Abstract: Commissural neurons send axons across the spinal cord midline and project to various supraspinal targets. These neurons exhibit diversity across multiple parameters, including their developmental origins, the transcriptional programs that drive their specification during embryogenesis, and their mature cell body positions, connectivities, molecular profiles, and functions. While much of this heterogeneity has been documented, the molecular programs that translate commissural neuron developmental characteristics into mature phenotypes remain poorly understood. Using novel genetic tools in mice, we are following subsets of commissural neurons from neurogenesis to adulthood with the goal of connecting developmental cell lineages, transcriptional trajectories, and mature properties. We are combining single-cell RNA sequencing, birthdating approaches, and viral circuit tracing methods to generate a detailed map of commissural neuron development and connectivity, with a special focus on spinal somatosensory circuits. Here we present early-stage data with respect to the diverse transcriptomes of embryonic commissural neuron subtypes and their connectivity patterns in the adult. Our studies promise to provide novel insight into the programs that govern developmental and mature diversity of commissural neurons, and these advances will enable further studies of individual commissural neuron subpopulations.

Disclosures: J. Abolafia: None. **A.J. Tulloch:** None. **D. Croote:** None. **A. Jaworski:** None.

Poster

196. Cell Lineage Analysis

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 196.11/A11

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH grant R01NS096240

Title: A progenitor-anchored fate mapping strategy

Authors: *M. M. PEREIRA LUPPI, J.-F. POULIN, G. CARONIA-BROWN, C. HOFER, P.-K. HSU, R. AWATRAMANI;
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Abstract: Lineage tracing techniques aim to identify the progeny of a defined population of progenitor cells. In the developing central nervous system, the neural tube undergoes morphogenetic changes while thousands of cells migrate long distances, complicating the analysis of progenitor-progeny relationships. Current lineage analysis relies on Cre recombinase to indelibly label a genetically defined progenitor population and its progeny. Such cumulative approaches are flawed by the fact that Cre driver gene is often expressed in both progenitors and postmitotic neurons, leading to ambiguous interpretation of the resulting fate map. Genetic inducible approaches temporally restrict the labeling of progenitor cells but are limited by mosaicism and toxicity. Here we develop and validate PRISM, an intersectional genetic approach where the Flp recombinase expression is both dependent on Cre, and restricted to neural progenitors. This approach can be used with all Cre lines to produce bona fide progenitor fate maps. We applied PRISM to resolve the lineage of progenitors expressing either of two developmentally important, but contentious lineages - Shh and Cux2.

Disclosures: M.M. Pereira Luppi: None. **J. Poulin:** None. **G. Caronia-Brown:** None. **C. Hofer:** None. **P. Hsu:** None. **R. Awatramani:** None.

Poster

196. Cell Lineage Analysis

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Topic: A.01. Neurogenesis and Gliogenesis

Support: Fondecyt Regular 1190848
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PIA-Conicyt ECM-12
Beca Conicyt N°21161386

Title: Analysis of the ciliary protein, IIIG9, reveals a new localization in cell-cell adhesion in ependymal cells and progenitor radial glial cells

Authors: *V. BAEZA[1], M. J. OVIEDO[1], F. A. MARTINEZ[1], M. CIFUENTES[2], F. J. NUALART[1], K. A. SALAZAR[1];
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Abstract: Introduction: In the adult brain, IIIG9 is restricted to the cilia present in ependymal cells that line the ventricular layer. During embryonic development (E14), a subset of radial glia generate ependymal cells, and ependymal cell differentiation is finalized after birth (P20). We have previously determined that IIIG9 expression is high towards the apical side of radial glia, although its function is unknown. We have characterized IIIG9 expression and localization during radial glia specification to ependymal cells in the rat brain, showing that is present in cell-cell adhesion-complexes through ependymal development.
Materials-Methods: We used embryonic rats (E13, E15, and E17) to analyze IIIG9 expression in the brain by RT-PCR and immunohistochemical analysis coupled to optical and spectral confocal microscopy. We used whole-mount staining of rat ventricular surface from E17 to adult to analyze the changes of IIIG9 distribution during specification and maturation from radial glia to ependyma. We also analyzed IIIG9 using SIM super-resolution microscopy and ultrastructural immunohistochemistry to localize IIIG9 in adult ependyma.
Results: IIIG9 is expressed throughout the ventricular wall during embryogenesis, and colocalizes with Pan-cadherin and β-catenin in the lateral membrane at the apical part of radial glial cells. In the transition from radial glia to ependymal cells, IIIG9 delocalizes from the cell-cell junctions. We demonstrated that IIIG9 is present in cell-cell adhesion junctions using SIM super-resolution and ultrastructural immunohistochemistry.
Discussion: IIIG9 may be involved in the maintenance of cell-cell adhesion complexes at the lateral membrane of radial glia that will generate ependymal cells in postnatal rats, giving this ciliary protein possible functions outside cilia and suggesting a novel role for IIIG9 during embryonic CNS development and in the adult postmitotic ependymal wall.

Disclosures: V. Baeza: None. **M.J. Oviedo:** None. **F.A. Martinez:** None. **M. Cifuentes:** None. **F.J. Nualart:** None. **K.A. Salazar:** None.

Poster

196. Cell Lineage Analysis

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Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH grant HD036379-20

Title: A novel subgroup of neurons related to the serotonergic neuronal system: Rhombomere 4-derived Pet1+ cells referred to as "para-serotonergic"

Authors: *Y. CHANG[1], B. OKATY[1], G. MADDALONI[1], M. RIEHS[2], R. HAYNES[2], S. DYMECKI[1];
[1]Genet., Harvard Med. Sch., Boston, MA; [2]Pathology, Boston Children's Hosp. and Harvard Med. Sch., Boston, MA

Abstract: The brain serotonergic (5-HT) neuronal system can be divided anatomically into rostral and caudal groupings. These two domains are separated by the rhombomere (r) 4-derived pontine region, which has been reported to harbor few 5-HTergic neurons. Notwithstanding this paucity of r4-derived 5-HT neurons, we and others (Okaty *et al*., 2019; Barrett *et al*., 2016; Pelosi *et al*., 2014; Alonso *et al*., 2013) have shown that transcripts encoding the 5-HTergic differentiation transcription factor *Pet1* are expressed within the r4 territory of the hindbrain. Here we show, using an intersectional genetic fate mapping strategy combined with histology and molecular approaches, that a substantial population of r4-Pet1 cells are located in the r4-derived pontine raphe nucleus, as well as some in the ventral portion of 5-HTergic B9 nucleus and rostral part of the Raphe Magnus; most of these neurons lack immunodetectable levels of 5-HT. We refer to these cells as "para-serotonergic," reflecting their lack of detectable 5-HT and their close spatial proximity to and common developmental specification with 5-HTergic neurons. Transgenically driving synaptophysin-GFP in these cells to illuminate axonal boutons, we found that r4-Pet1 para-serotonergic neurons selectively innervate the parabrachial nucleus, pre-Bötzinger complex, the nucleus of the solitary tract, and the hypoglossal nucleus. These findings suggest a role in the control and coordination of physiological functions including breathing and heart rate. We are currently working on molecular characterization of r4-Pet1 para-serotonergic neurons using single cell RNAseq and physiological functional studies.

Disclosures: Y. Chang: None. **B. Okaty:** None. **G. Maddaloni:** None. **M. Riehs:** None. **R. Haynes:** None. **S. Dymecki:** None.

Poster

196. Cell Lineage Analysis

Location: Hall A

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Program #/Poster #: 196.14/A14

Topic: A.01. Neurogenesis and Gliogenesis

Title: Lineage-based organization of the motor system in *Drosophila* larvae

Authors: *Y.-W. WANG, C. C. WREDEN, J. L. MENG, Z. D. MARSHALL, E. S. HECKSCHER;
Univ. of Chicago, Chicago, IL

Abstract: One of the long-standing goals in neuroscience has been to rewire neuronal circuits using stem cells for therapeutic purposes. Much effort has been made to uncover the neuronal stem cell lineage-based principles underlying circuit assembly. For example, stem cell lineage and birth timing of neurons can contribute to synaptic wiring decisions in some, but not all, parts of the central nervous system. Little is known about the lineage-based organization of the motor system, yet such knowledge is a prerequisite for stem cell-based repair of spinal cord injury. We propose three lineage-based motor circuit assembly models. First, neurons from the same stem cell precursor could be more likely to form synapses with each other, which we call "lineage matching". Second, groups of neurons from certain lineages, but not other lineages, could prefer to wire together, which we call "preferential lineage assortment". Third, neurons from different lineages that are born at the same time could preferentially wire-together, which we call "birth time matching". METHODS: We test the above models using neuronal stem cells, neuroblasts (NBs), in the model system, *Drosophila melanogaster*. In particular, we focus on the NB3-3 lineage, one of the best characterized lineages in the *Drosophila* motor system. First, we identify the synaptic inputs onto neurons derived from NB3-3 using information in the *Drosophila* larval connectome (a TEM volume spanning the entire larval CNS). Second, we apply twin spot MARCM, a lineage tracing and birth time dating transgenic tool developed in *Drosophila*, to determine the birth order of both the neurons of the NB3-3 lineage and of neurons which synapse onto these neurons. These two tools have never before been applied to the same set of neurons, and here will provide us novel insights into the lineage-based development of motor circuits by allowing us to distinguish between lineage-based models of circuit assembly. RESULTS: I will discuss the recent progress in the meeting. DISCUSSION: By revealing relationships between lineage and circuitry in motor system of *Drosophila*, our work will better the understanding of circuit assembly, and may contribute to circuit engineering for therapeutic medicine.

Disclosures: Y. Wang: None. **C.C. Wreden:** None. **J.L. Meng:** None. **Z.D. Marshall:** None. **E.S. Heckscher:** None.

Poster

196. Cell Lineage Analysis

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 196.15/A15

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant RO1NS082262

Title: Map3k12 binding inhibitory protein (Mbip) function establishes the appropriate number of cortical interneurons

Authors: *C. A. SMOOT[1], K. A. WASEF[2], E. S. TUCKER[1];
[1]Neurosci., [2]Biol., West Virginia Univ., Morgantown, WV

Abstract: Inhibitory interneurons exert powerful control over cerebral cortex activity, and deficits in their function have been heavily implicated in the etiologies of autism spectrum disorder, epilepsy, and schizophrenia. Many of these deficits have been attributed to aberrant interneuron development, occurring well before the onset of disease. The objective of this study is to assess the role of Map3k12 binding inhibitory protein (*Mbip*) as a regulator of cortical interneuron development *in vivo*. *Mbip* expression is enriched in the medial ganglionic eminence (MGE) of the developing forebrain, where most cortical interneurons are generated. To determine the *in vivo* function of *Mbip*, we developed a novel mouse model in which we have conditionally removed *Mbip* from cortical interneuron progenitor cells of the MGE. We found a significant decrease in the density of reporter-labeled MGE-derived cortical interneurons within the postnatal cortex in our conditional knockout mice compared to heterozygous littermate controls. We next evaluated the requirement for *Mbip* in establishing the two primary MGE-derived interneuron subtypes, parvalbumin (PV) and somatostatin (SOM) interneurons. We found a significant decrease in the density of reporter and PV co-labeled cortical interneurons. Preliminarily, we also observe a decrease in reporter and SOM co-labeled cortical interneurons. Together, our results support a cell-autonomous role for *Mbip* in establishing the appropriate number of MGE-derived cortical interneurons within the postnatal cortex. Current efforts are aimed at determining whether the loss of MGE-derived interneurons is uniform across cortical layers and areas, and whether compensatory changes in non-targeted populations of cortical interneurons occur. We are also investigating developmental mechanisms underlying the loss of MGE-derived cortical interneurons in *Mbip* conditional knockout mice in order to determine how interneuron deficits emerge in this model. Future work will investigate the functional consequences of *Mbip* loss on animal behavior and cortical electrophysiology. This research will further our understanding of cortical circuitry formation and may provide insight for the development of future therapeutic approaches for cortical circuitry disorders.

Disclosures: C.A. Smoot: None. **K.A. Wasef:** None. **E.S. Tucker:** None.

Poster

196. Cell Lineage Analysis

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 196.16/A16

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant R01NS082262

Title: The multifaceted roles for JNK in forebrain development

Authors: *J. G. CUNNINGHAM, S. NTI, J. SCRIPTER, E. S. TUCKER;
Neurosci., West Virginia Univ., Morgantown, WV

Abstract: Proper function of forebrain circuitry is necessary for a wide variety of behavioral tasks such as the perception of sensory stimuli and the orchestration of body movements. Execution of these tasks depends on the precise communication between different brain regions, whose interconnectivity is established during embryonic development. Indeed, disruptions to normal brain development can lead to long-lasting changes in the adult brain, resulting in disorders such as schizophrenia. In previous work from our lab, we demonstrated a role for the c-Jun N-terminal Kinase (JNK) signaling pathway in the migration of cortical interneurons. To study the requirement for JNK *in vivo*, we developed a conditional triple knockout (cTKO) mouse model where *Jnk1* is deleted from *Dlx5/6*-lineage cells, which are located in the ventral telencephalon and a portion of the diencephalon, in mice lacking both *Jnk2* and *Jnk3*. In the current study, we find that the same mutation leads to disruptions in non-interneuronal populations of cells, as well as major axon pathways. Interestingly, many of these disruptions occur in areas not targeted by the conditional deletion of *Jnk1*, suggesting that there are nonautonomous requirements for JNK signaling in forebrain development. We have analyzed *in vivo* cortices ranging from embryonic (E) day 12.5 to postnatal day 0. In cTKO brains, gross changes include enlarged ventricular volume, malformations of the developing cortical plate, and misrouted thalamocortical axons. Axon pathway defects are detectable at E12.5 and persist throughout embryonic development. Additionally, structures within the ventral forebrain such as the striatum and globus pallidus appear malformed and hypo-morphic. By characterizing the generation and maturation of different forebrain regions throughout embryonic development, we will further define the roles of the three JNK genes in the morphogenesis of the brain. Understanding the genetic regulation of brain development will help uncover potential causes of neurodevelopmental disorders, and can ultimately lead to better treatment of these devastating diseases.

Disclosures: J.G. Cunningham: None. **S. Nti:** None. **J. Scripter:** None. **E.S. Tucker:** None.

Poster

196. Cell Lineage Analysis

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 196.17/A17

Topic: A.01. Neurogenesis and Gliogenesis

Support: Uehara memorial foundation
Naito foundation

Title: A novel-genome integrating vector system for direct transgenesis in neural stem cells

Authors: *T. KUMAMOTO[1], F. MAURINOT[1], R. BARRY[1], C. VASLIN[2], S. VANDORMAEL- POURNIN[3], M. LE[1], M. COHEN-TANNOUDJI[3], A. REBSAM[1], K. LOULIER[1], S. NÉDELEC[2], S. TOZER[1], J. LIVET[1];

[1]Inst. De La Vision – Sorbonne Univ., Paris, France; [2]Inst. du Fer à Moulin, Paris, France; [3]Inst. Pasteur, Paris, France

Abstract: In developmental neuroscience, direct transfection of neural stem cells in vivo with genome-integrating vectors is increasingly used as an alternative to the generation of transgenic animals. However, current genome-integrative vectors present important limitations: retroviral vectors traditionally used in cell lineage studies are cumbersome to produce and limited in cargo capacity. Naked DNA vectors are considerably simpler to use and can accept large transgenes, but they enable expression from non-integrated episomes in addition to that of integrated transgenes. This creates a transient burst of expression that prevents reliable identification of transgenic cells and can cause transgene leakiness and toxicity.
We bypass this issue with a novel expression strategy termed “iOn” (integration-coupled On expression) switch, enabling to activate DNA transgenes introduced by standard transfection procedures as they integrate into the host genome, while episomal transgenes remain silent. Toxic and non-physiological effects due to high episomal expression are thus avoided. Marker expression solely reflects the activity of integrated transgenes, enabling straightforward identification and analysis of integration events directly after transfection.
We demonstrate the efficiency of the iOn strategy for stable additive transgenesis in neural progenitors in the embryonic cortex, retina and spinal cord of mice and chicken. In these systems, we show that iOn vectors coupled with fluorescent color reporters make an ideal tool for cell lineage tracing and open the way to functional mosaic analysis through simple and rapid somatic transfection procedures. iOn vectors also drive leak-proof genome integration-dependent Cre-lox recombination, which we use to reveal the clonal output of a genetically identified subtype of retinal progenitors in the developing chick retina. We also show that iOn vectors provide an efficient means to stably transfect and rapidly isolate clones of stem/pluripotent cells in vitro. These results establish iOn as a highly efficient and versatile strategy for “direct transgenesis” in any model accessible to transfection.

Disclosures: **T. Kumamoto:** None. **F. Maurinot:** None. **R. Barry:** None. **C. Vaslin:** None. **S. Vandormael- Pournin:** None. **M. Le:** None. **M. Cohen-Tannoudji:** None. **A. Rebsam:** None. **K. Loulier:** None. **S. Nédelec:** None. **S. Tozer:** None. **J. Livet:** None.

Poster

196. Cell Lineage Analysis

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 196.18/A18

Topic: A.03. Stem Cells and Reprogramming

Title: Direct conversion of microglia into neurons improves neurological recovery after ischemic injury

Authors: *T. IRIE[1,2], T. MATSUDA[1], Y. HAYASHI[3], J.-I. KIRA[2], K. NAKASHIMA[1]; [1]Stem Cell Biol. and Med., [2]Neurol., Grad. Sch. of Med. Sciences, Kyushu Univ., Fukuoka, Japan; [3]Aging Sci. and Pharmacol., Fac. of Dent. Science, Kyushu Univ., Fukuoka, Japan

Abstract: Stroke including brain ischemia, a major cause of death in developed countries, is usually associated with severe disabilities, high recurrence rate and other poor outcomes. Since neuronal loss is a major pathological hallmark of brain injury, regenerating new neurons to replenish lost neurons at injured sites is considered to be critical for functional recovery. However, treatments for stroke are currently restricted to symptomatic therapies, and no longer-term remedy has been established to date. Microglia, the major immune cells in the central nervous system, are known to converge at injured sites for the clearance of dead cells. We have recently revealed that a single transcription factor, NeuroD1 (ND1), can convert mouse microglia into neurons in the adult mouse striatum. With this background, we examined whether microglia-neuron conversion at lesion sites can induce functional recovery after ischemic brain injury. Eight-week-old mice were subjected to 30 minutes of middle cerebral artery occlusion (MCAO) by an intraluminal suture. We found that microglia intruded into and accumulated in/around the lesion site by 7 days after MCAO. In order to express ND1 in the microglia, we injected lentivirus expressing ND1 under the control of CD68 promoter into the lesion site of the striatum 7 days after MCAO, and observed neuronal marker-positive induced neuronal (iN) cells 2 and 4 weeks after the virus injection. When we depleted microglia specifically from the brain before the virus injection by the treatment with PLX5622, an inhibitor of colony-stimulating factor-1 receptor, ND1-converted iN cells could be hardly observed, suggesting that majority of original cells converted into iN cells was microglia. We also detected excitatory synaptic formation of iN cells with host neurons in the injured striatum and these iN cells exhibited spontaneous action potential firing following depolarization. Furthermore, these stroke model mice with ND1-induced neuronal conversion of microglia exhibited significantly improved functional recovery at 4 weeks after viral injection and afterwards. Taking these results into account, it is conceivable that our findings advanced one step toward the development of therapeutic strategy for patients afflicted with sequelae of stroke.

Disclosures: T. Irie: None. **T. Matsuda:** None. **Y. Hayashi:** None. **J. Kira:** None. **K. Nakashima:** None.

Poster

196. Cell Lineage Analysis

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 196.19/A19

Topic: A.02. Postnatal Neurogenesis

Title: Pioneer factor NeuroD1 rearranges transcriptional and epigenetic profiles to execute neuronal conversion from microglia

Authors: *T. MATSUDA[1], T. IRIE[1], S. KATSURABAYASHI[2], Y. HAYASHI[3], K. NAKASHIMA[1];
[1]Dept. of Stem Cell Biol. and Med., Grad. Sch. of Med. Sciences, Kyushu Univ., Fukuoka, Japan; [2]Fukuoka Univ., Fukuoka, Japan; [3]Dept. of Aging Sci. and Pharmacol., Fac. of Dent. Science, Kyushu Univ., Fukuoka, Japan

Abstract: Lineage-specific transcription factors enable the switch from one cell type into another, with potential applications in disease modeling and regenerative therapy. Recent studies indicate that exogenous Sox2 or NeuroD1 (ND1) expression converts endogenous astrocytes in the mouse brain and spinal cord to neurons. Since glial cells including astrocytes proliferate in response to injury in the central nervous system (CNS) and eventually form a glial scar, in vivo neuronal conversion from astrocytes raises the possibility of modifying gliotic tissues to provide a cellular source for replenishing impaired neuronal circuits. Microglia, the major immune cells in the CNS, also converge at injured sites and become a predominant cell type within the glial scar. Furthermore, a recent report has shown that even after selective elimination of most microglia (>99%) in the adult mouse brain, the population can be rapidly replenished from the few surviving microglia (<1%). Thus, microglia that have accumulated at injured sites should be suitable for restoring lost neurons by direct conversion, without exhaustion of the source in the brain. Nevertheless, the direct conversion of microglia into neurons has not been achieved. Here, we show that NeuroD1 achieves direct neuronal conversion from mouse microglia both in vitro and in vivo. Exogenous NeuroD1 initially occupies closed chromatin regions associated with bivalent H3K4me3 and H3K27me3 marks in microglia to induce neuronal gene expression. These regions are resolved to a monovalent H3K4me3 mark at later stages of the reprogramming to establish neuronal identity. Furthermore, the transcriptional repressors Scrt1 and Meis2 are induced as NeuroD1 target genes, resulting in a decrease in the expression of microglial genes. In parallel, the microglial epigenetic signature in promoter and enhancer regions is erased. These findings reveal NeuroD1 pioneering activity accompanied by global epigenetic remodeling for two sequential events: the onset of neuronal property acquisition and the loss of microglial identity during the reprogramming.

Disclosures: T. Matsuda: None. **T. Irie:** None. **S. Katsurabayashi:** None. **Y. Hayashi:** None. **K. Nakashima:** None.

Poster

196. Cell Lineage Analysis

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 196.20/A20

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant NS100514

Title: Limited NG2 glial tropism of recombinant adeno-associated viral (rAAV)-mediated gene delivery for *in vivo* neuronal reprogramming

Authors: *M. THAQI[1], E. REISENBIGLER[2], R. GREENE[2], R. A. MARR[1], D. A. PETERSON[2];
[1]Neurosci., Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL; [2]Ctr. for Stem Cell & Regenerative Med., Rosalind Franklin Univ. Med. Sci., North Chicago, IL

Abstract: Direct reprogramming of cell identity from a glial to neuronal phenotype has been demonstrated both *in vitro* and *in vivo*. The induction of phenotypic neurons from glial cell populations follows forced expression of pioneering transcription factors, such as Ngn2, NeuroD1, and Ascl1 that are normally expressed during development to specify neuronal fate. Viral vectors can be used to deliver reprogramming transcription factors to glial cells. The efficiency of vector tropism has a major impact on the feasibility to reprogram a sufficient number of new neurons to achieve a meaningful functional integration. NG2 glia, also known as oligodendrocyte progenitor cells (OPCs), represent one cellular population that could be an ideal target for neuronal induction for repair. NG2 glia, understood to represent a reserve cell population to replace oligodendrocytes, are actively dividing in the mature CNS, perform no known critical neural activity and respond to injury by proliferation and subsequent population homeostasis. Previously, we have successfully targeted and reprogrammed NG2 glia to neurons *in vitro* and *in vivo* using retroviral vectors to target the actively proliferating NG2 cell population. As an alternative delivery platform, rAAV vectors are frequently used to achieve efficient and widespread *in vivo* gene delivery. However, no systematic assessment of AAV serotype efficiency in targeting NG2 glia has been reported. Therefore, we investigated this concept by delivering CMV-eGFP constructs into rat cortex and striatum (n=3 animals per site) using the following AAV serotypes: 1, 2, 4, 5, 6, 6.2, 8, 9, rh10, DJ, PHP.S, PHP.B, PHP.eB. Analysis of infected cell types, three weeks post-injection, revealed most serotypes were substantially neurotropic with tropism for astrocytes also observed with AAV5 and AAV8. However, cultured adult-rat derived NG2 glia were infected by AAV 6.2 at remarkably high efficiency, followed by the AAV PHP variants B, eB and S suggesting that these serotypes may be suitable for *in vivo* gene delivery to NG2 glia populations in the CNS. Interestingly, NG2 glia from different brain regions, such as motor cortex, corpus callosum, and striatum, exhibited biological heterogeneity in transduction efficacy between different AAV serotypes. This may be due to developmental differences of tangential migration in sequential waves of NG2 glia generation during development. Ultimately, this comprehensive AAV serotype study elucidates the transduction efficiency of individual serotypes for NG2 glia on distinct brain regions.

Disclosures: **M. Thaqi:** None. **E. Reisenbigler:** None. **R. Greene:** None. **R.A. Marr:** None. **D.A. Peterson:** None.

Poster

196. Cell Lineage Analysis

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 196.21/A21

Topic: A.03. Stem Cells and Reprogramming

Support: IBS institutional Grant (IBS-R001-D1)
Creative Research Initiative Program, Korean National Research Foundation (2015R1A3A2066619)

Title: Transdifferentiation of reactive astrocytes into functional neurons causes motor recovery after spinal cord injury

Authors: *H. AN;
Inst. for Basic Sci. (IBS), Daejeon, Korea, Republic of

Abstract: Transdifferentiation is a promising therapy for replacing dead neurons in spinal cord injury (SCI) where scar-forming reactive astrocytes proliferate robustly, allowing themselves as a prime target for reprogramming into neurons. In this study, we directly reprogrammed reactive astrocytes into functional neurons with particular molecular tools, comprising a double-floxed transcription factor, neurogenin-2 (Ngn2) and a split-Cre under two different promoters for reactive astrocytes specific gene expression. In the injured mouse brain, over 60% of virally-transfected reactive astrocytes transdifferentiated into NeuN positive neuron-like cells with robust action potential via expressing Ngn2. In the AAV-EF1a-df-Ngn2-IRES-GFP with split cre infected SCI (SCI/Ngn2) group, motor impairment was significantly recovered to 34% of the normal level in Basso mouse score (BMS), consistent with showing action potential. Ngn2-expressing cells colocalize with neuronal markers but not with BrdU, indicating that they are originated not from cell-division, but from the cell-fate switch. These results show that reactive astrocytes directly convert to neurons without risk of tumorigenesis caused by stemness of infected cells. Our study proposes the transdifferentiation of reactive astrocytes into functional neurons as a next-generation therapeutic approach for patients suffering from spinal cord injury.

Disclosures: H. An: None.

Poster

197. Developmental Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 197.01/A22

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant R00NS089943

Title: Loss of PCDH12 causes cell migration and differentiation defects in human embryonic stem-cell derived neuroprogenitors

Authors: *J. M. RAKOTOMAMONJY, L. E. RYLAARSDAM, D. A. THOMAS, S. L. MCDERMOTT, G. LANGUREN, A. D. GUEMEZ-GAMBOA;
Physiol., Northwestern University, Feinberg Sch. of Med., Chicago, IL

Abstract: Protocadherin (PCDH)12 is a cell adhesion molecule, member of the cadherin superfamily, that mediates homophilic cell-cell interactions. Loss of PCDH12 was previously shown to result in microcephaly, facial dysmorphism, epilepsy, and developmental disability. How PCDH12 loss of function leads to malformations of cortical development such as microcephaly, and disorders associated with disrupted neuronal circuitry such as epilepsy is unknown. Here, we investigated the effects of PCDH12 deletion in neuroprogenitors derived from human embryonic stem cells. We showed that PCDH12 absence affects cell migration. Neuroprogenitors lacking the cadherin failed at migrating as far as wild-type (WT) cells due to disrupted directional persistence. PCDH12 knock-out cells did not show any proliferative defect or change in cell death rate. However, we observed a decrease in cell cycle re-entry when compared to WT neuroprogenitors, suggesting a premature exit from the progenitor state and early neuronal differentiation. Our data suggest that abnormal cell migration and fate determination could be responsible for the disrupted cortical development and neuronal circuitry observed in patients carrying homozygous PCDH12 variants. These results provide insight into the cellular mechanisms regulated by PCDH12 during brain development.

Disclosures: J.M. Rakotomamonjy: None. **L.E. Rylaarsdam:** None. **D.A. Thomas:** None. **S.L. McDermott:** None. **G. Languren:** None. **A.D. Guemez-Gamboa:** None.

Poster

197. Developmental Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 197.02/A23

Topic: A.01. Neurogenesis and Gliogenesis

Support: 17H03895
17K19500
17K08281

Title: Reelin regulates the positioning of late-born neurons during hippocampal development

Authors: *M. HATTORI, **K. ISHII**, T. KOHNO;
Grad. Sch. Pharmaceuti. Sci., Nagoya City Univ., Nagoya, Japan

Abstract: Introduction: Reelin is a large secreted protein and essential for brain formation and functions. Secreted Reelin binds to its receptors, apolipoprotein E receptor 2 (ApoER2) and very low-density lipoprotein receptor (VLDLR), and induces the tyrosine phosphorylation of Dab1. Phosphorylated Dab1 activates downstream signaling and then is degraded. We previously reported that the C-terminal region (CTR) of Reelin is required for efficient phosphorylation of Dab1. In the knock-in mouse in which the CTR of Reelin is deleted (ΔC-KI mouse), hippocampal CA1 layer was split into two layers (Kohno et al. J. Neurosci. 35, 4776 (2015)). Furthermore, ΔC-KI mice exhibited hyperactivity and impaired working memory (Sakai et al. Sci. Rep. 6, 28636 (2016)). These abnormalities suggested the specific role of Reelin CTR in hippocampal formation and functions. In this study, we aim to clarify the significance of the CTR of Reelin during hippocampal development.
Methods: The amount of Dab1 was analyzed by western blotting. The hippocampal neuron generated at embryonic day (E) 12.5 or 16.5 were labeled using bromodeoxyuridine (BrdU). Frozen hippocampal sections of wild-type and ΔC-KI mice were immunostained with anti-Ctip2 and anti-BrdU antibodies. To observe neuronal morphology, we transfected immunofluorescent expression vector to hippocampal neurons by *in utero* electroporation.
Results and Discussion: The amount of Dab1 in the ΔC-KI mice was greater than that of wild type mice suggesting that Reelin-Dab1 signaling is attenuated in the hippocampus of ΔC-KI mice. In the ΔC-KI mice, the CA3 layer appeared normal, but the CA1 pyramidal cell layer was split into two layers. This defect was not observed at postnatal day (P) 0 but was apparent at P3and afterward. In ΔC-KI mice injected with BrdU at E12.5, BrdU positive cells were correctly positioned in the CA1 layer. On the other hand, in ΔC-KI mice injected with BrdU at E16.5, BrdU-positive cells were

Disclosures: **M. Hattori:** None. **K. Ishii:** None. **T. Kohno:** None.

Poster

197. Developmental Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 197.03/A24

Topic: A.01. Neurogenesis and Gliogenesis

Support: JSPS KAKENHI 17K07428
Grant-in-Aid for Scientific Research on Innovative Areas: 「Interplay of developmental clock and extracellular environment in brain formation」 19H04795
Takeda Science Foundation Research Grant 2018
The Naito Foundation Research Grant 2018

Title: Extracellular matrix reorganization in the subplate layer is important for the regulation of radial neuronal migration in the developing neocortex

Authors: *N. KANEKO[1,2], N. MAEDA[2], K. YURA[1,3], C. OHTAKA-MARUYAMA[2]; [1]Life Sci., Ochanomizu Univ., Tokyo, Japan; [2]Neural Network, Tokyo Metropolitan Inst. of Med. Sci., Tokyo, Japan; [3]Advanced Sci. and Engin., Waseda Univ., Tokyo, Japan

Abstract: In the developing neocortex, newborn neurons initially show multipolar (MP) morphology, moving slowly with no particular direction (MP migration). When MP neurons reach the subplate (SP) layer, they change into bipolar (BP) shape (MP-to-BP transition), and initiate fast radial glial fiber-dependent locomotion (locomotion) toward pial surface. This radial neuronal migration process occurs in a birth- date-dependent inside-out manner, leading to the generation of the six layered structure of neocortex. Previously, we performed gene expression profiling of migrating excitatory neurons, and found that the expressions of genes related to extracellular matrix (ECM) are significantly changed during migration. Among these genes, we focused on ADAMTS2 (a disintegrin and metalloproteinase with thrombospondin motifs2), the expression of which remarkably increased during migration. Both siRNA-mediated knockdown and overexpression of ADAMTS2 in migrating neurons disturbed MP-to-BP transition, suggesting that theproper levelsof ADAMTS2are required or the initiation of locomotion. Recently, it has been reported that TGF-β signaling-related proteins arepotential proteolytic substratesof ADAMTS2. It has also been reported that TGF-β initiates signaling pathways to fate naive neurites into axons in the early neocortical neurons.However, the involvement of TGF-β signaling in radial neuronal migration remains to be clarified. Immunohistochemistry of pSmad3, which is one of the activated downstream protein of TGF-β signaling,revealed that pSmad3 is expressed just under the subplate, Thus, we hypothesized that ADAMTS2 regulates neuronal migration throughtransient activation of TGF-β signaling at the SP. In order to investigate the involvement of TGF-β signaling in neuronal migration, we monitored TGF-β/ pSmad signaling

during neuronal migrationusing cultured corticalslices by luminescent imaging. The results suggested that TGF-β signaling is up-regulated in the migrating MP neurons just before switching to the locomotion mode. We are now analyzing the downstream pathways of TGF-β signaling inradial migration process including the regulation of F-actin dynamics.

Disclosures: **N. Kaneko:** None. **N. Maeda:** None. **K. Yura:** None. **C. Ohtaka-Maruyama:** None.

Poster

197. Developmental Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 197.04/A25

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant DC012441
NIH Grant DC015438
NIH Grant DC013791

Title: Development of mouse olfactory tubercle as part of the ventral striatum

Authors: *E. MARTIN-LOPEZ[1], C. XU[2], T. LIBERIA-VAYA[1], S. J. MELLER[1], C. A. GREER[1];
[1]Neurosurg., Yale Univ. Sch. of Med., New Haven, CT; [2]Harvard Med. Sch., Cambridge, MA

Abstract: The olfactory tubercle (OT) has been traditionally considered a 3-layer paleocortical structure receiving direct input from the olfactory bulb. However, anatomically and histologically the OT resembles the striatum (ST), and its function and connectivity with the dopaminergic reward system led to the suggestion that the OT be considered ventral striatum (vST). Here, in an analysis of OT development, we provide evidence that OT and vST share developmental origins and features. Neurogenesis analyses with BrdU established that OT follows a lateral-to-medial maturation gradient. Using a piggyBac transposon as either a multi- or a single-color technique, we characterized the migratory route followed by the OT neuroblasts. From their origin in the lateral ganglionic eminence (LGE) they migrate to the OT using a pathway we named “ventral migratory course” (VMC). In the OT neuroblasts differentiated, acquiring ST medium-spiny neurons (MSN) molecular phenotype. Of interest, the fate or laminar distribution of cells in the OT was determined in part by the progenitor cell of origin. Additionally, we studied the dopaminergic innervation of the OT using tyrosine hydroxylase (TH) staining. The first TH^+ axons arrived in the OT at E13 and progressively innervated the OT in an inside-out manner. Postnatally, we determined the myelination of OT fibers occurred in “rapid myelination process” between P7-P14 as occurs in piriform cortex. Collectively, both our developmental analyses of neuroblast origin/migration and molecular

characterization of cells as they mature in the OT, are consistent with the notion that the OT should be considered as part of the vST.

Disclosures: E. Martin-Lopez: None. **C. Xu:** None. **T. Liberia-Vaya:** None. **S.J. Meller:** None. **C.A. Greer:** None.

Poster

197. Developmental Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 197.05/A26

Topic: A.01. Neurogenesis and Gliogenesis

Title: Alk inactivation disrupts neurodevelopment of cerebral organoids

Authors: *Y. KONG, R. MAO;
Southeast Univ., Nanjing, China

Abstract: Proper neurodevelopment of the mammalian cortex is critically dependent upon a series of highly orchestrated complex events, including proliferation and differentiation of neural progenitor cells, neural migration and maturation. In this study, we aim to transiently and specifically disrupt the neurodevelopment in cerebral organoids derived from human ESCs by manipulating anaplastic lymphoma kinase (ALK), a receptor-type protein tyrosine kinase. *ALK* is specifically expressed in the embryonic central nervous system. However, its physiological roles, particularly in the context of mammalian brain development, remain poorly understood. Our results show that the transient pharmacological inactivation of ALK significantly impaired proliferation of neural progenitor cells and delayed the development of cerebral organoids. To gain mechanistic insights, we employed single-cell RNA-sequencing (scRNA-seq) to unbiasedly dissect the cell composition and transcriptional heterogeneity and identified molecular signaling pathways and differentially expressed genes (DEGs) that are affected by ALK inhibition in human cerebral organoids. Our results provide compelling evidence supporting a crucial role of ALK in mammalian neurodevelopment.

Disclosures: Y. Kong: None. **R. Mao:** None.

Poster

197. Developmental Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 197.06/A27

Topic: A.01. Neurogenesis and Gliogenesis

Support: ZIA NS002824-28

Title: Drebrin regulates cytoskeletal dynamics in migrating GnRH neurons through interaction with CXCR4

Authors: *Y. SHAN[1], S. WRAY[2];
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Abstract: Coordination of extracellular guidance cues and intracellular cytoskeletal elements is required for migration of neurons to the correct location, and subsequent formation of functional circuits. Chemokine stromal cell-delivered factor 1 (SDF-1), together with its receptor chemokine receptor type 4 (CXCR4) are important regulators of migration in both the nervous system and immune system. SDF-1 via CXCR4 is known to promote Gonadotropin releasing hormone-1 (GnRH) neuronal migration. However, how CXCR4/SDF-1 activation alters cytoskeletal components in neurons, remains unclear. Developmentally regulated brain protein (drebrin) stabilizes actin polymerization and interacts with microtubule plus ends. Recently, Perez-Martinez et al. proposed that drebrin directly interacted with CXCR4 to promote actin activities at immune synaptic sites in T-cells. The current study examined whether CXCR4, under SDF-1 stimulation, interacts with drebrin to facilitate neuronal migration in GnRH cells during embryonic development. At E12.5 and E14.5, robust drebrin expression was identified along cortical actin in migratory GnRH neurons, and drebrin was colocalized with the microtubule plus end binding protein EB1, consistent with the role of drebrin guiding microtubules into cortical actin. Confocal microscopy showed colocalization between drebrin and CXCR4. Using migration assays on GnRH cells maintained in nasal explant, blocking drebrin (using BTP2, a Ca^{2+} release-activated Ca^{2+} channel inhibitor) prevented the effects of CXCR4 agonist/antagonist on neuronal movement, consistent with a functional interaction between drebrin and CXCR4. In GnRH leading processes, STED microscopy revealed that the association between EB1 and drebrin decreased after BTP2 treatment and co-immunoprecipitation confirmed there was a direct interaction between CXCR4 and drebrin in mouse brain and an immortalized GnRH cell line. Analysis of Drebrin KO animals is in progress to determine if movement of GnRH neurons is perturbed. These data show a novel mechanism by which a chemokine via a membrane receptor communicates with the intracellular cytoskeleton components in migrating neurons during early nervous system development.

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

Poster

197. Developmental Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 197.07/A28

Topic: A.01. Neurogenesis and Gliogenesis

Support: R01AA021981
OD011092

Title: Orientations of cellular processes in rhesus macaques during the second half of gestation for biomechanical simulations of cerebral cortical folding

Authors: X. WANG[1], K. E. GARCIA[2], P. V. BAYLY[3], ***C. D. KROENKE**[1];
[1]Oregon Hlth. & Sci. Univ., Portland, OR; [2]Indiana Univ., Evansville, IN; [3]Washington Univ., St. Louis, MO

Abstract: Abnormal gyral and sulcal folding patterns are observed in individuals affected by neurodevelopmental disorders of heterogeneous, and in many cases unknown etiology. A biomechanical understanding of folding would be of utility for interpreting the folding pattern of a mature brain in terms of cellular developmental processes critical to the mechanism of disease. Over the folding period, dramatic brain growth accompanies the transformation of its structural organization from developmentally transient lamina, to a set of white matter fiber bundles that interconnect the cytoarchitecturally differentiated cerebral cortex and other brain areas. Tissue growth and deformation depends on the orientations and mechanical properties of its cellular constituents. Recently, we have developed computational methods to simulate anisotropic growth. For example, axon elongation along the direction of a developing fiber tract occurs in response to mechanical tension. Here we report the laminar position dependence of anisotropy in radial glial cell, dendritic, and axonal processes in the fetal rhesus macaque occipital lobe from gestational day 90 (G90) to G135. At G90, prior to the formation of folds, SMI312-stained axons are oriented radially within the cortical plate (CP), they are uniformly-distributed within the subplate (SP), oriented parallel to the ventricular and pial surfaces within the outer fibrous layer (oFL), and are uniformly-distributed within the subventricular zone (SVZ). In contrast, radial glial cell processes stained with vimentin exhibit strong radial orientation within the CP, SP, and oFL. MAP2-stained dendrites appear radially oriented in CP and SP, and appear to be randomly oriented within the oFL and SVZ. These cell process orientation patterns are unchanged at G110, during the initial period of cortical folding. By G135, primary sulci and gyri have formed, and the SVZ and oFL are diminished in volume. Radial alignment of vimentin and MAP2-positive cell processes are observed, but SMI312-stained axons do not adopt a strongly preferred orientation in the CP. In the subjacent developing white matter, the predominant orientation of axons depends on the location relative to a gyral crown or sulcal fundus. These findings indicate

that the CP is primarily composed of radially-oriented cell processes over the course of cerebral cortical folding, but within subcortical lamina, particularly the oFL, the preferred orientations of different cellular elements are highly heterogeneous. This will enable more detailed modeling of tissue deformation in response to forces generated in the growing brain over the period of cerebral cortical folding.

Disclosures: **X. Wang:** None. **K.E. Garcia:** None. **P.V. Bayly:** None. **C.D. Kroenke:** None.

Poster

197. Developmental Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 197.08/A29

Topic: A.01. Neurogenesis and Gliogenesis

Support: NRF-2012R1A5A2048183
NRF-2018R1A2B6006131

Title: Jak3 modulates the migration of GABAergic interneurons during development of murine cortex

Authors: *A. KIM[1,2], J. CHUNG[3,2], E. BAIK[3,2];
[1]Physiol., Ajou Univ., Suwon, Korea, Republic of; [2]Chronic Inflammatory Dis. Res. Ctr., [3]Physiol., Ajou Univ. Sch. of Med., Suwon, Korea, Republic of

Abstract: The proper function of cerebral cortex requires a balanced and coordinated network with the excitatory glutamatergic projection neurons and the inhibitory GABAergic interneurons. During development, these different types of neurons are originated from spatially and molecularly segregated progenitors and are moved along the distinct migratory pathways. Among several types of neurons, interneurons navigate along multiple tangential migration routes to settle into appropriate developing cortical layers. Disturbed neuronal migration gives rise to neurological or neuropsychiatric disorders, such as congenital epilepsy, autism spectrum disorder, and schizophrenia. Here, we identified JAK3 as a modulator of migration and differentiation of interneurons during developmental stage. More than 70% of interneurons are produced in medial ganglionic eminence (MGE) and move to the developing cortex in mouse embryonic day 13.5 to 15.5. In the present study, we found the JAK3 expression of the lateral migrating stream of interneurons in the E13.5 and E15.5 embryonic brain was prominent. In *ex vivo* slice culture also, interneurons from MGE explant moved to the cortex explant, however, inhibition of JAK3 delayed the tangential migration of interneurons toward the developing cortex. In *in vitro* neuroprecursor cell cultures from MGE in E13.5 mice, MGE-derived interneurons could cross the scratched space in wound-healing, and pharmacological or genetic inhibition of JAK3 significantly decreased the migration of interneurons. These effects may be

shown by controlling extracellular cues, such as chemoattraction and extracellular matrix organization. These results suggest the possibility that JAK3 is a proper modulator of migration of GABAergic interneurons from MGE to cortex during corticogenesis.
This work was supported by the Chronic Inflammatory Disease Research Center (NRF-2012R1A5A2048183) and NRF-2018R1A2B6006131.

Disclosures: **A. Kim:** None. **J. Chung:** None. **E. Baik:** None.

Poster

197. Developmental Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 197.09/A30

Topic: A.01. Neurogenesis and Gliogenesis

Support: NS002824-29

Title: Ccdc141, a genetic factor of Kallmann syndrome: Isoforms are differently localized in GnRH cells

Authors: *H. CHO[1], S. WRAY[2];
[1]NIH/NINDS, Bethesda, MD; [2]Cell. & Developmental Neurobio., NIH NINDS, Bethesda, MD

Abstract: Development of Gonadotropin releasing hormone-1 (GnRH) neurons is important for a functional reproduction system in vertebrates. Disruption of GnRH results in idiopathic hypogonadotropic hypogonadism (IHH) and if accompanied by anosmia is termed Kallmann Syndrome (KS). From their origin in the nasal placode, GnRH neurons migrate along olfactory-derived vomeronasal axons to the nasal forebrain junction and then turn caudally into the developing forebrain. Research on the origin of GnRH neurons, their migration and genes associated with KS have identified multiple factors which influence development of this system. *CCDC141* is one of the most recently identified genes to be involved in IHH. Five different mutations in the *CCDC141* gene have been found from both of nIHH and KS patients. Previous studies showed that 1) Ccdc141 was expressed in migrating GnRH neurons and that 2) knockdown of *Ccdc141* using siRNA reduced GnRH neuronal migration but not olfactory axon outgrowth. To further investigate the role of Ccdc141 *in vivo,* we obtained and analyzed *Ccdc141* transgenic mice with a *LacZ* reporter gene followed by a stop codon that replaced exon 3 and 4 of *Ccdc141*. Unexpectedly, we found splicing occurred in these transgenic mice that resulted in altered (deletion of exon 3 and 4) Ccdc141 proteins. The phenotype of this line of *Ccdc141* transgenic mice was similar to WT with respect to reproductive function and GnRH cell migration, suggesting that the region corresponding to exon 3 and 4 is not functionally essential and/or that other Ccdc141 isoform(s) compensate for the Ccdc141-exon3/4 protein. RACE PCR analysis revealed three transcriptional *Ccdc141* isoforms in mice and

immunofluorescence showed these isoforms to be differentially localized in an immortalized GnRH cell line (NLT), one cytoplasmic and two nuclear. Analysis of *Ccdc141* transgenic mice also suggested that there is a direct correlation in transcriptional expression between *Ccdc141* and its neighboring gene, *Ttn*, especially in heart. We are currently generating a new *Ccdc141* KO mouse line that targets all three isoforms to determine the role of Ccdc141 in neuronal migration and pathogenic mechanism of IHH, as well as its functional interaction with Ttn.

Disclosures: H. Cho: None. **S. Wray:** None.

Poster

197. Developmental Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 197.10/A31

Topic: A.01. Neurogenesis and Gliogenesis

Support: IBRO-SfN Travel Grants

Title: The physiological role of the GTPase Rab21 in neuronal migration and the development of the cerebral cortex

Authors: S. DUPRAZ[1], M. PEROTTY[2], J. BUSTOS PLONKA[2], S. QUIROGA[2], ***L. J. SOSA**[2]; [1]Axonal Growth and Regeneration, German Ctr. for Neurodegenarative Diseases,, Bonn, Germany; [2]CIQUIBIC-CONICET Depto. Química Biológica FCQ-UNC, Cordoba, Argentina

Abstract: The development of the complex structure of the mammalian neocortex requires the proper migration of developing neurons from the ventricular zone containing neural progenitors to the cortical plate. The precise coordination of different cellular processes such as cytoskeleton dynamics, membrane trafficking, and cell adhesion during migration is achieved by a variety of signaling pathways. GTPases play a central role in all these processes. In this context, the small GTPase Rab21 has been implicated in the regulation of cell adhesion dynamics by controlling the trafficking of endocytic vesicles containing adhesion molecules. Interestingly, Rab21 has been also implicated in neurite outgrowth. In preliminary experiments, we expressed dominant negative Rab21(T31N) in the embryonic cortex by *in utero* electroporation (IUE). In contrast to controls, neurons expressing Rab21(T31N) were arrested within the VZ/SVZ, indicating an important function for Rab21 in neuronal migration. Notably, the neurons that fail to enter the cortical plate showed increased neurite branching and multipolar morphology. These studies are important to better understand the mechanism governing the development of the cerebral cortex and the mechanisms that participate in neurodevelopmental pathologies such as autism spectrum disorders and cortical malformations.

Disclosures: **S. Dupraz:** None. **M. Perotty:** None. **J. Bustos Plonka:** None. **S. Quiroga:** None. **L.J. Sosa:** None.

Poster

197. Developmental Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 197.11/A32

Topic: A.01. Neurogenesis and Gliogenesis

Support: TAMU Internal Funds

Title: The role of astroglial sphingosine-1-phosphate receptor 1 in the developing brain

Authors: *P. SHETH[1], J. LI[1,2];
[1]Dept. of Vet. Integrative BioSciences, [2]Texas A&M Inst. for Neurosci., Texas A&M Univ., College Station, TX

Abstract: Sphingosine 1-phosphate (S1P), a bioactive lipid molecule, regulates diverse biological processes including cell proliferation, migration, and activity through a family of G-protein coupled S1P receptors (S1PRs). Among S1PRs, S1PR1 is highly expressed in the central nervous system (CNS); however, its physiological function in the CNS remains poorly understood. *In situ* hybridization analysis of mouse brains at various postnatal developmental stages reveal abundant and increased expression of S1PR1 in Bergmann glia of the cerebellum and protoplasmic astrocytes of the grey matter overtime. We hypothesize that S1PR1 mediated signaling contributes to normal brain development and that disruption of astrocytic S1PR1 may result in functional alterations of Bergmann glia and consequently in differential cell migration, cell proliferation, and cerebellar functionality. To investigate the functions of astroglial S1PR1 *in vivo*, we generated conditional *Aldh1l1*^{CreERT2}*:S1pr1*$^{fl/fl}$ mice where *S1pr1* is selectively ablated in astrocytes, including Bergmann glia, in a tamoxifen-inducible manner. Immunohistochemistry analysis reveal strong immunoreactivity and membranous localization of S1PR1 in Bergmann glial processes from wild-type mice but not in those from the conditional knockout mice. We are currently employing double immunohistochemistry and EdU tracing methods to determine the effects of *S1pr1* ablation on proliferation and maturation of Bergmann glia and on migration of granule cells over the first two weeks of postnatal cerebellar development. We will further examine the role of S1PR1 in development of astrocytes using Ai14 reporter mice to visualize all astrocytes that underwent *S1pr1* deletion. This study shall provide new insights into the *in vivo* functions of S1PR1 in astroglial development and their interactions with neurons.

Disclosures: **P. Sheth:** None. **J. Li:** None.

Poster

197. Developmental Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 197.12/DP01/A33

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: A.01. Neurogenesis and Gliogenesis

Support: RO1-NS080928
RO1-NS098372
T32-GM07507

Title: Role of F-BAR proteins in process extension and neuronal migration in the embryonic cortex

Authors: *R. J. TAYLOR[1], J. CARRINGTON[2], L. GERLACH[1], E. W. DENT[1];
[1]Neurosci., Univ. of Wisconsin Madison, Madison, WI; [2]Univ. of Wisconsin, Madison, WI

Abstract: Neurite initiation from a newly born neuron is a critical step in neuronal differentiation and migration. We have previously described opposing roles of two closely related membrane bending F-BAR proteins, Formin-Binding Protein 17 (FBP17) and Cdc42-Interacting Protein 4 (CIP4) in neurite initiation in primary cortical neurons. FBP17 expression results in prominent endocytic tubulation of the plasma membrane, filopodia formation and precocious neurite outgrowth. Conversely, CIP4 expression results in protrusion of lamellipodia-like veils, which inhibit filopodia formation and neurite initiation. Thus, the opposing roles of FBP17 and CIP4 in neurite formation are the result of two types of plasma membrane alterations, endocytic tubulation and protrusion. Here, we expand upon this finding *in vivo*, utilizing a novel approach to label and manipulate neurons via *in utero* electroporation. We have validated a plasmid system (Double UP) which allows the user to generate two populations of differently colored cells. In the absence of manipulation, these cells are distinguishable only by color (i.e. red and green). However, genetic overexpression or knockdown can be linked to cells of only one color (i.e. red), allowing for simultaneous visualization of both control and experimental cells in an individual cortical slice. Using this technique, we are determining the role of both endocytic tubulation and membrane protrusion in neuronal migration and differentiation. CIP4 expression in the developing cortex peaks very early in prenatal development and falls to almost undetectable levels at birth. We show that when CIP4 expression is maintained at high levels it inhibits cortical neuron migration in the developing cortex. Currently, we are determining the mechanism by which CIP4 expression inhibits migration and what effects FBP17 overexpression and knockdown have on cortical neuron migration. Results from this study will provide fundamental insights into how proteins that induce endocytic tubulation or membrane protrusion

function to regulate cortical neuron migration and process outgrowth in the early developing mammalian brain.

Disclosures: **R.J. Taylor:** None. **J. Carrington:** None. **L. Gerlach:** None. **E.W. Dent:** None.

Poster

197. Developmental Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 197.13/A34

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH T32-NS076401
NIH T32-DC00011

Title: Poly (ADP-ribose) polymerase 1 (PARP1) regulates Reelin expression in the embryonic brain

Authors: M. M. NELSON[1], D. R. GRAYSON[2], *G. CORFAS[1];
[1]Kresge Hearing Res. Inst., The Univ. of Michigan, Ann Arbor, MI; [2]Psychiatry, Univ. of Illinois at Chicago, Chicago, IL

Abstract: Reelin (Reln) is a protein secreted by Cajal-Retzius cells, the earliest born neurons in the embryonic cortex. Reln binds to receptors in newborn neurons and regulates their migration. Accordingly, animals that lack or highly overexpress Reln have defects in cortical layering. Despite the critical functions of Reln in brain development, the mechanisms regulating its expression remain poorly understood. We found that the enzyme Poly (ADP-ribose) Polymerase 1 (PARP1) regulates Reln expression in the embryonic brain and neural precursor cells (NPCs) in culture. PARP1 is an enzyme that acts by adding ADP-ribose polymers (PAR) to proteins, including itself, using NAD as a substrate. This post-translational modification called PARylation results in changes in the activity of its substrate proteins and has been shown to impact processes such as transcription and DNA repair. Interestingly, mutations in genes that affect PARylation have been associated with cognitive dysfunction and neurodegeneration. Several studies have also demonstrated that PARP1 regulates transcription of several neuronal genes *in vitro*, e.g. *DCX*, *DYX1C1*, and *MASH1*. Yet, the effects of PARP1 in gene expression in the embryonic brain have not been studied. To define the impact of PARP1 in gene expression during cortical development, we performed RNA sequencing of E15.5 mouse cortex from wild types and PARP1 knockouts (KOs) (n = 4 for each genotype). We identified 48 altered genes, with most of them (n = 41) being upregulated, suggesting that PARP1 primarily acts as a repressor in this tissue. Gene ontology analysis indicated that these genes are relevant to neuronal migration and cell adhesion. *Reln* was among the most upregulated transcripts in the PARP1 KOs, and Western blot analysis showed upregulation of Reln protein levels at the same

age (n=6 wild type and KOs). RT-qPCR analysis of NPCs in culture from both wild types and PARP1 KOs showed that *Reln* over-expression also occurs *in vitro*, suggesting it is a cell autonomous process. Furthermore, we found that treatment with a PARP1 inhibitor increases *Reln* mRNA levels in cultured NPCs (n=4 independent experiments). Interestingly, a luciferase-based assay showed that inhibition of PARP1's enzymatic activity in NPCs does not increase the activity of the *Reln* promoter (n = 3 independent experiments), suggesting that the role of PARP1 is not just to repress *Reln* transcription. Importantly, PARP1 KO mice do not present with obvious defects in cortical layering or cell proliferation. Future studies will further explore the mechanisms by which PARP1 regulates *Reln* mRNA levels and potential subtle changes in brain architecture in PARP1 KOs.

Disclosures: M.M. Nelson: None. **D.R. Grayson:** None. **G. Corfas:** None.

Poster

197. Developmental Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 197.14/A35

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant 1R01HD097331-01
NIH Grant 1R15HD09641101

Title: Gli3 loss-of-function leads to impaired GnRH-1 neuronal migration to the brain

Authors: *E. M. TAROC[1], J. M. LIN[1], A. NAIK[1], E. GENIS[1], G. FUCHS[1], D. KEEFE[2], R. BALASUBRAMANIAN[2], P. E. FORNI[1];
[1]Biol., Univ. at Albany, Albany, NY; [2]Reproductive Endocrine Unit, Massachusetts Gen. Hosp., Boston, MA

Abstract: Gonadotropin releasing hormone (GnRH-1) neurons (ns) play a central role in controlling the reproductive axis of vertebrates by releasing GnRH-1 to induce the release of gonadotropin during key phases throughout development and life. GnRH-1ns are generated in the developing vomeronasal organ and migrate to the brain along the projection of the terminal nerve. Defects in GnRH-1 neuronal migration or signaling can lead to various forms of congenital hypogonadotropic hypogonadism (HH) that translate to the absence of puberty and sterility. In humans, HH can clinically manifest as either: (i) Kallmann syndrome (KS), wherein HH is associated with an impaired sense of smell; or (ii) normosmic idiopathic hypogonadotropic hypogonadism (nIHH), wherein HH occurs with a normal sense of smell. Since HH can appear with and without a normal sense of smell strongly suggest an oligogenic nature for these disorders.
Gli3 is a downstream effector molecule of sonic hedgehog (Shh) signaling, where it will act as a

transcriptional activator or repressor depending on the presence or absence of Shh respectively. It has been shown that mutations in Gli3 can cause a spectrum of craniofacial defects and disrupt the development of the olfactory system. Interestingly it was also shown that knocking down Gli3 can cause defective migration of the GnRH-1ns in mice.
Here we examined the development of the vomeronasal sensory neurons (VSNs), terminal nerve (TN) and GnRH-1ns, in a Gli3 knockout mouse model [Gli3 extra-toe (Xt)]. Our study reveals that Gli3 loss of function leads to defective neurogenesis of the VSNs which is most likely mediated by the dramatic loss of the Ascl-1 expressing progenitors. Characterization of the GnRH-1ns in $Gli3^{Xt/Xt}$ showed an almost complete absence of GnRH-1ns in the brain, and dramatic misrouting for TN and vomeronasal neurons. The brains of the Gli3 knockout mice also showed alterations in the expression of the guidance cue Semaphrin-3A.
We tested if an Ascl1 knockout can recapitulate the Gli3 knockout phenotype. In Ascl-1 mutants we found dramatic reduction for both VSNs and GnRH1-ns. This suggest that Ascl-1 expression is crucial for the neurogenesis of both populations, but GnRH-1 neurogenesis is independent of Gli3 activity.
Analyzing whole exome data from a large cohort of nIHH/KS human patients, we identified several new rare GLI3 variants. Loss of function for the identified variants was confirmed via luciferase assays. We propose that human GLI3 mutations could play an important modifier role in KS/nIHH.

Disclosures: **E.M. Taroc:** None. **J.M. Lin:** None. **A. Naik:** None. **E. Genis:** None. **G. Fuchs:** None. **D. Keefe:** None. **R. Balasubramanian:** None. **P.E. Forni:** None.

Poster

197. Developmental Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 197.15/A36

Topic: A.01. Neurogenesis and Gliogenesis

Support: PHS Grant R21MH108994
Brain Health Institute, Rutgers University

Title: Developmental changes in microglial populations

Authors: *J. J. GIFFORD, I. MULLEN, M. KARTHIKEYAN, G. C. WAGNER, A. W. KUSNECOV;
Psychology, Rutgers Univ., New Brunswick, NJ

Abstract: Microglia are the primary immunocompetent cells of the brain and have recently been a focus of neurodevelopmental research. Among various recognized functions, they serve as a surveillance system, serving two primary roles: synaptic pruning and phagocytosis. The present

study examined the baseline development of murine microglia from the early postnatal period through adulthood. Time points included postnatal days P8, P15, P30 and P60, focusing on the prefrontal cortex (PFC), hippocampus, and cerebellum. Results indicated a developmentally regulated increase in microglia counts in the PFC, but a decline in hippocampal microglia by adulthood. Specifically, microglia counts increased by 44% in the PFC between P8 and adulthood, while hippocampal counts decreased by 54% between P15 and adulthood. These data extend previous results by Kim et al. (2015) in which increased microglial counts were observed solely in the hippocampus between P10 and P15, followed by a decline. This study provides foundation data for assessing the response of microglia to apoptosis induced in these regions by postnatal treatment with valproic acid (VPA). It is anticipated that there will be increased numbers of microglia observed in areas surrounding apoptotic cells of VPA-treated animals compared to saline controls. Collectively, these data may shed light on the underlying mechanism through which postnatal VPA causes neurobehavioral disruption akin to autistic regression (Wagner et al., 2006).

Disclosures: **J.J. Gifford:** None. **I. Mullen:** None. **M. Karthikeyan:** None. **G.C. Wagner:** None. **A.W. Kusnecov:** None.

Poster

197. Developmental Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 197.16/A37

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIHR01NS082262-01

Title: JNK signaling coordinates the dynamic behavior of migrating cortical interneurons

Authors: ***S. E. HICKLING**[1], N. COKER[3], K. KEEN[2], E. S. TUCKER[2];
[1]Biochem. and Mol. Biol., [2]West Virginia Univ., Morgantown, WV; [3]Bates Univ., Lewistown, ME

Abstract: During corticogenesis, inhibitory interneurons must migrate into the developing cerebral cortex, deposit in the correct cortical layer, and establish connections with their appropriate partners. Aberrant migration of inhibitory interneurons can alter the formation of cortical circuitry and lead to several neurological disorders including epilepsy, autism and schizophrenia. Cortical interneurons initially travel in tangentially oriented streams to enter and migrate through the cerebral cortex, and then turn radially to invade the cortical plate. Our lab previously found that disruption of the c-Jun NH_2-terminal kinase (JNK) signaling pathway delayed the entry of interneurons into the cortex, as well as led to the premature departure of cortical interneurons from migratory streams. In the current study, we used live-cell confocal

microscopy to explore the mechanisms by which JNK activity coordinates two cell biological processes that are essential for the guided migration of cortical interneurons: nucleokinesis and leading process branching. Nucleokinesis is a cyclical process whereby the cell bodies of migrating cortical interneurons translocate into a cytoplasmic swelling formed in their leading process. We found that pharmacological inhibition and genetic ablation of JNK-signaling in cortical interneurons impairs nucleokinesis by decreasing translocation distances and increasing pause time between translocation events. Moreover, we found that JNK signaling controls the subcellular localization of the centrosome and primary cilium, two organelles involved in nucleokinesis. In JNK-inhibited cells, both centrosomes and cilia spend significantly more time in the soma and trailing process than in controls. These findings suggest that JNK plays a major role in the cellular control of migration through nuclear movement. To orient their direction of migration, cortical interneurons extend and retract leading process branches to respond to chemotactic guidance cues present in their environments. We found that upon JNK-inhibition, the stability of leading process branches is affected with decreased frequency of growth cone splits, and shorter branch duration. Together, these data indicate that JNK controls multiple aspects of cell migration in cortical interneurons. Current efforts are aimed at unraveling the mechanism through which JNK controls interneuron migration by exploring JNK's role in cytoskeletal dynamics and chemotaxic attraction to gradient cues found in the cortex. Our results will improve our understanding of cortical development and hopefully provide novel insight into the etiology of cortical circuit disorders.

Disclosures: S.E. Hickling: None. **N. Coker:** None. **K. Keen:** None. **E.S. Tucker:** None.

Poster

197. Developmental Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 197.17/A38

Topic: A.01. Neurogenesis and Gliogenesis

Support: NSF-DBI (1126118)
HHMI Undergraduate Research Grants

Title: Chlorpyrifos-treated *Xenopus laevis* show abnormal neural development of non-cholinergic spinal sensory neurons

Authors: *K. DEGNER, M. BRYSON, J. HIDALGO LOPEZ, A. WARD, E. HERRERA, F. WATSON;
Washington and Lee Univ., Lexington, VA

Abstract: Chlorpyrifos (CPF), an organophosphate pesticide (OP) commonly used in agriculture, kills insects by inhibiting acetylcholinesterase (AChE), an enzyme that normally

hydrolyzes acetylcholine (ACh) at the synapse. This accumulation of ACh in the synapse results in overstimulation of the postsynaptic neuron. OPs accumulate in ground water and contaminate food, impacting secondary non-target species such as humans and amphibians. The effects of CPF on cholinergic neurons leads to physiological and morphological abnormalities, but its effect on non-cholinergic neurons is not well established. Here, we characterize a *Xenopus laevis* frog line expressing green fluorescence protein (GFP) in sensory spinal neurons identified as Rohon-Beard neurons and dorsal root ganglia. CPF-treated (ranging from 0 - 10 μM) *Xenopus* embryos collected at stages 37 and 47, two stages corresponding with peak neural development, show decreased numbers of sensory neurons and increased migratory defects that vary as a function of CPF concentration. Our results characterize the severity of CPF-induced neuronal abnormalities and provide evidence that CPF exposure affects non-cholinergic neuronal development.

Disclosures: K. Degner: None. **M. Bryson:** None. **J. Hidalgo Lopez:** None. **A. Ward:** None. **E. Herrera:** None. **F. Watson:** None.

Poster

198. Synaptogenesis and Activity-Dependent Development II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 198.01/A39

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

Support: FWF Grant P27031
FWF Grant W1101

Title: Presynaptic differentiation at the neuromuscular junction requires $Ca_V1.1$-driven calcium signaling

Authors: *M. M. KAPLAN, B. E. FLUCHER;
Med. Univ. Innsbruck, Innsbruck, Austria

Abstract: Proper development of synapses requires reciprocal communication between presynaptic neurons and their postsynaptic target cells. At the neuromuscular junction (NMJ), nerve-induced regulation of the postsynaptic specialization is well-studied, whereas retrograde mechanisms, by which the muscle controls the presynaptic differentiation, are still poorly understood. Recently we have shown that $Ca_V1.1$-driven calcium signals regulate the correct organization of postsynaptic AChR clusters during NMJ formation. Here we utilized two genetic mouse models, both of which lack $Ca_V1.1$-driven calcium signals, to demonstrate a central role of activity-induced skeletal muscle calcium signaling in the retrograde regulation of presynaptic differentiation of the NMJ. In mice lacking $Ca_V1.1$ expression and thus activity-dependent calcium signals correct fasciculation and navigation of the motor nerves were perturbed during

early NMJ development. Axons failed to recognize their termination territory and motor axon endings grew beyond postsynaptic AChR clusters. Moreover, in the absence of postsynaptic activity-dependent calcium signaling neurites failed to differentiate specialized nerve terminals. Together these observations strongly suggest that postsynaptic muscle calcium signaling functions upstream of multiple retrograde pathways to induce comprehensive differentiation of the motor neurons.

Disclosures: M.M. Kaplan: None. **B.E. Flucher:** None.

Poster

198. Synaptogenesis and Activity-Dependent Development II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 198.02/A40

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

Support: NIMH RO1 MH 041083
NIMH 5T32 MH019524
NIMH T32 MH 963314

Title: Molecular mechanisms underlying specificity of synapse formation in cultured neurons of *Aplysia*

Authors: *A. ALEXANDRESCU, T. J. CAREW;
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Abstract: The formation of specific neural connections is critical for the proper development of the nervous system, and shares mechanistic similarities with the formation and strengthening of synapses during memory formation. Here we test the general hypothesis that developmental and learning-related plasticity engage similar molecular mechanisms. The marine mollusk *Aplysia californica* is a powerful model system for studying the cellular and molecular mechanisms underlying both of these forms of plasticity. In *Aplysia*, facilitation of monosynaptic connections between identified presynaptic sensory neurons (SNs) and postsynaptic motor neurons (MNs) contributes significantly to memory for sensitization. Moreover, the SN-MN microcircuit demonstrates developmental synapse-specificity and can be reconstituted in culture, offering single-cell spatial resolution for the examination of the molecular mechanisms underlying the generation of specific neural connections. *In vitro*, as *in vivo*, *Aplysia* SNs selectively form chemical synapses with their physiological target MNs, but do not form synapses either with themselves or with non-target MNs. Using neuronal co-cultures, pharmacological manipulations, and single-cell analyses, we investigated the molecular mechanisms governing the interactions between SNs and target and non-target MNs, focusing on three evolutionarily-conserved molecular processes: i) endogenous growth factor signaling, ii) transcription, and iii) activation

of protein kinases. We show that SNs have differential effects on target and non-target MNs and that, surprisingly, SNs exert an inhibitory transcriptional effect on non-target MNs. Our results suggest that the effects of SNs on non-target MNs require paracrine signaling from the SNs as well as autocrine signaling from the MNs. Taken together these findings describe a novel form of regulation of molecular signaling between mismatched synaptic partners, supporting a general model in which the inappropriate postsynaptic MN is actively rejected by molecular signaling from the presynaptic SN. Our current experiments are aimed at investigating the effects of these molecular changes on synaptic structure and function.

Disclosures: A. Alexandrescu: None. **T.J. Carew:** None.

Poster

198. Synaptogenesis and Activity-Dependent Development II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 198.03/A41

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

Support: Brain & Behavior Research Foundation NARSAD 27662

Title: Investigating the role of astrocytes in the development of synaptic connectivity in a rodent model of neonatal abstinence syndrome

Authors: *T. C. BOGGESS[1], A. MAZUR[1], H. SEXTON[1], W. C. RISHER[2];
[2]Biomed. Sci., [1]Marshall Univ., Huntington, WV

Abstract: Neonatal abstinence syndrome (NAS) has become a major health concern in the United States and Central Appalachia in particular as a result of the widespread opioid epidemic, but remarkably little is known about the long-term effects of prenatal opioid exposure on brain development. Recently, Marshall University physicians have noted a specific clinical presentation of NAS (involving tongue thrusting, wandering eye movements, and exaggerated Moro reflex) in infants prenatally exposed to opioids and gabapentin, a drug commonly given for the treatment of pain and seizure. Gabapentin is also known to inhibit the development the synaptic pathways in the brain by interfering with secreted proteins (i.e. thrombospondins) from astrocytes, prompting us to hypothesize that the pathology of NAS is dependent on impaired astrocyte synaptogenic signaling in the developing brain.
In this study, we developed a mouse model of NAS using mice transgenic for the thrombospondin/gabapentin receptor, α2δ-1, so that the effects of co-abuse of the opiate buprenorphine and gabapentin on synaptic development could be examined. First-time pregnant dams were given daily access to buprenorphine (5 mg/kg) and gabapentin (30 mg/kg) in a condensed milk mixture from gestational day 7 until approximately 11 days following the birth of their litter. Control dams were fed a vehicle control condensed milk mixture. The pups were

then sacrificed at postnatal day 21. Brains were harvested and fixed for immunohistochemistry. Brain cryosections including addiction related areas (prefrontal cortex [PFC], anterior cingulate cortex [ACC] and nucleus accumbens [NAc]) were cut and then stained for excitatory and inhibitory synaptic markers. At this timepoint, we found that heterozygous α2δ-1 mice had significantly increased excitatory synapse number in the ACC and NAc with a concomitant decrease in synapses in the PFC following combined prenatal buprenorphine/gabapentin exposure. These same mice had significantly increased inhibitory synapse number in the ACC with a concomitant decrease in synapses in the PFC and NAc.
Ongoing experiments will determine whether the extent of this synaptic reorganization is shifted in an α2δ-1-dependent manner, further implicating astrocyte involvement in the pathology of NAS.

Disclosures: **T.C. Boggess:** None. **A. Mazur:** None. **H. Sexton:** None. **W.C. Risher:** None.

Poster

198. Synaptogenesis and Activity-Dependent Development II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 198.04/A42

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

Support: N_HKUST625/15
HKUST10/CRF/12R
HKUST12/CRF/13G
CUHK2/CRF/11G
T13-607/12R
AoE/M-05/12
AoE/M-604/16

Title: Ubiquitination of neuroligin2 controls dynamics of inhibitory synapse formation

Authors: Z. LI, H. TIAN, J. XIA;
Div. of Life Sci., Hong Kong Univ. of Sci. and Technol., Clear Water Bay, Kowloon, HongKong, China

Abstract: Human brain is made of billions of neurons that are connected by synapses. Neuronal activity has been reported to play an important role in the generation and disappearance of synapses. However, the mechanism underling the ever-changing synaptic dynamics is still unclear. Neuroligin2 (NL2), a major inhibitory synaptic compartment, has been found to be crucial for inhibitory synapse formation and functions. Deletions or mutations in NL2 are also associated with several brain disorders such epilepsy, autism and schizophrenia. Here, we report that TTX-induced neuronal activity blockage modifies the ubiquitination level of NL2, resulting

in a decreased inhibitory synapse density. In addition, ubiquitination of NL2 was found to be crucial for inhibitory synapse stability. To characterize the mechanism for this, we performed an immunoprecipitation coupled mass spectrometry (IP-MS) experiments and identified a potential candidate which controls ubiquitination and further modulates inhibitory synaptogenesis. Our study provides novel insights into the physiological mechanism for the dynamic process of synapse formation and regulation.

Disclosures: Z. Li: None. **H. Tian:** None. **J. Xia:** None.

Poster

198. Synaptogenesis and Activity-Dependent Development II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 198.05/A43

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

Support: CURE innovator award
NARSAD Distinguished investigator award funded by the Hofmann Trust
Tuberous Sclerosis Alliance research grant

Title: RAB27A coordinates synaptic integration and sensory responses of cortical neurons via extracellular nanovesicle signaling

Authors: *L. ZHANG, X. ZHANG, T. LIN, S. TEAW, T. LAM, A. BORDEY;
Yale Sch. of Med., New Haven, CT

Abstract: RAB27A is an evolutionarily conserved small GTPase that regulates vesicle trafficking and displays copy-number variants associated with increased risk of autism. However, the function of RAB27A on brain development is unknown. Here, we identified a novel form of paracrine communication between populations of developing neurons that involves RAB27A-mediated extracellular nanovesicle signaling. In the developing somatosensory cortex, we show that decreasing RAB27A levels selectively in upper-layer neurons did not affect synaptic integration of these neurons, but it increased glutamatergic synaptic transmission in barrel cortical neurons and responses to whisker stimulation. This effect involved an age-dependent transfer of the epigenetic regulator HDAC2 via extracellular nanovesicles that coordinated the development of dendritic spines across neuronal populations. Thus, we found that a RAB27A-dependent nanovesicle-based communication coordinates the development of synaptic connectivity among neuronal populations shedding light on how a small Rab GTPase tunes circuit development and responses to sensory stimulation.

Disclosures: L. Zhang: None. **X. Zhang:** None. **T. Lin:** None. **S. Teaw:** None. **T. Lam:** None. **A. Bordey:** None.

Poster

198. Synaptogenesis and Activity-Dependent Development II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 198.06/A44

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

Support: NINDS/NIH intramural research program

Title: A conserved tyrosine residue in Slitrk3 carboxyl-terminus is critical for GABAergic synapse development

Authors: J. LI, W. HAN, K. WU, X. GU, L. ZHOU, Y. LI, Q. LIU, ***W. LU**;
NINDS/NIH, Bethesda, MD

Abstract: Single-passing transmembrane protein, Slitrk3 (Slit and Trk-like family member 3, ST3), is a synaptogenic cell adhesion molecule highly expressed at inhibitory synapses. Recent studies have shown that ST3, through its extracellular leucine-rich repeat (LRR) domains, selectively regulates inhibitory synapse development via the trans-synaptic interaction with presynaptic cell adhesion molecule, receptor protein tyrosine phosphatase δ (PTPδ) and the cis-interaction with postsynaptic cell adhesion molecule, Neuroligin 2 (NL2). However, little is known about the physiological function of ST3 intracellular, carboxyl (C)-terminal region, in synapse development. Here we report that in heterologous cells, ST3 C-terminus is not required for ST3 homo-dimerization and trafficking to the cell surface. In contrast, in hippocampal cultured neurons, the conserved tyrosine Y969 (in mice) in ST3 C-terminus is critical for GABAergic synapse development. Indeed, overexpression of ST3 Y969A mutant markedly reduced the gephyrin puncta density and GABAergic transmission in hippocampal neurons. In addition, CRISPR-Cas9-based single-cell genetic deletion of ST3 strongly impaired GABAergic transmission both *in vitro* and *in vivo*. Importantly, wild-type (WT) ST3, but not the ST3 Y969A mutant, could fully rescue GABAergic transmission deficits in neurons lacking endogenous ST3, confirming a critical role of Y969 in the regulation of inhibitory synapses. Taken together, our data identify a single critical residue in ST3 that is important for GABAergic synapse development and function, and highlight the importance to understand the unique signaling mediated by ST3 C-terminus in inhibitory synaptogenesis.

Disclosures: J. Li: None. **W. Han:** None. **K. Wu:** None. **X. Gu:** None. **L. Zhou:** None. **Y. Li:** None. **Q. Liu:** None. **W. Lu:** None.

Poster

198. Synaptogenesis and Activity-Dependent Development II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 198.07/A45

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

Support: NICHD Intramural Research Program HD008962

Title: At the end of the brainbow: Dissecting chandelier cell connectivity

Authors: M. ISAAC, B. NJERI, Y.-J. ZHANG, ***T. J. PETROS**;
NICHD, Bethesda, MD

Abstract: Why do cortical interneurons synapse on only a subset of pyramidal cells within their axonal arbor? What are the mechanisms that regulate this connectivity logic? Understanding these complex interactions is extremely challenging considering that most interneurons can synapse on multiple regions of a postsynaptic cell with significant variability in connection probability. However, one population of GABAergic interneurons presents a unique opportunity to explore this connectivity logic. Chandelier cells (ChCs, also called axo-axonic cells) are a subtype of PV+ fast-spiking cells that selectively target the axon initial segment (AIS) of pyramidal cells in the cortex and hippocampus via specialized 'cartridges' of terminal boutons. The majority of cortical ChC cell bodies are located at the layer I/II boundary with their axonal projections extending into layers II/III. On average, a ChC synapses on ~20% of AIS within its axonal arbor, and each AIS receives input from ~3-5 ChCs. But the extent of ChC global connectivity logic remains unknown. Do ChCs form a specific innervation pattern in which particular subsets of cartridges from overlapping ChC axonal arbors consistently synapse on the same AIS? Or are ChC connections essentially random? To answer these questions, we are utilizing Brainbow mice and AAV viruses in which Cre expression drives recombination of multiple fluorescent cassettes such that each individual cell expresses a unique spectral signature. By analyzing overlapping, uniquely-labeled ChC axonal arbors, we can determine if these cells preferentially target the same AISs, distinct AISs or if their targeting is random. We are labeling ChCs using *PV-Cre* and *Nkx2.1-CreER* mice with tamoxifen injections at E16.5-E18.5. We have established protocols for performing Brainbow and AIS immunostaining in concert with Expansion Microscopy (ExM) to image ChC synaptic boutons in cleared brain tissue using lower magnification objectives. This set of experiments will provide significant insight into the connectivity logic of how ChCs target pyramidal neurons.

Disclosures: M. Isaac: None. **B. Njeri:** None. **Y. Zhang:** None. **T.J. Petros:** None.

Poster

198. Synaptogenesis and Activity-Dependent Development II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 198.08/A46

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

Title: The role of TMEM132B in inhibitory synapse development and function

Authors: *D. D. CASTELLANO, W. HAN, J. LI, K. WU, T. LI, W. LU;
NINDS/NIH, Bethesda, MD

Abstract: TMEM132 proteins are a family of five neural adhesion molecules that have recently been implicated in several neuropsychiatric disorders. These molecules are single-pass transmembrane proteins containing a cohesion domain, three BIG domains, a phosphatase-1 interaction motif, and a WAVE regulatory complex interacting receptor cytoplasmic motif. However, the roles of these proteins in the regulation of synapse development and function remain largely unknown. Recently, a proteomic screen has identified TMEM132B as a putative binding partner for $GABA_A$ receptors, suggesting a possible involvement of TMEM132B in inhibitory synapse regulation. To this end, we have generated a TMEM132B knockout mouse line and have employed electrophysiological and cell biological approaches to characterize synaptic phenotypes in hippocampal neurons.

Disclosures: D.D. Castellano: None. **W. Han:** None. **J. Li:** None. **K. Wu:** None. **W. Lu:** None. **T. Li:** None.

Poster

198. Synaptogenesis and Activity-Dependent Development II

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

Support: Fondo Nacional de Desarrollo Científico y Tecnológico Fondecyt Inicio N°11161056
Programa de Apoyo a Centros con Financiamiento Basal AFB 17004 to Fundación Ciencia y Vida

Title: Chaperone mediated autophagy modulates synaptic differentiation in hippocampal neurons

Authors: J. HORMAZÁBAL[1], L. YANTEN[1], J. DE LA CRUZ[1], N. MARTINEZ[1], A. O. ARDILES[2], ***I. E. ALFARO**[1];
[1]Lab. of Lysosome Biol. and Autophagy, Fundacion Ciencia Y Vida, Santiago, Chile;
[2]Neurociencia, Ctr. Interdisciplinario De Neurociencia De Valpa, Valparaiso, Chile

Abstract: Development of neuronal synapses is characterized by a coordinated assembly of proteins at pre-and post-synaptic regions. Protein homeostasis is crucial for the proper development, function and plasticity of synapses. Both local protein synthesis and degradation by the proteasome and lysosomes are important to maintain synaptic proteostasis. Chaperone-mediated autophagy (CMA) is a selective lysosomal protein degradation pathway characterized by the direct translocation of soluble cytosolic proteins to lysosomes through membrane pores formed by the lysosomal transmembrane protein, LAMP2A. CMA substrates contains KFERQ-like motifs important for the association with the HSC70 chaperone which assists in the recognition and transport through LAMP2A. Using bioinformatics analysis, we identified that 31.9% and 60.2% proteins from the pre- and post-synaptic mammalian proteome contain CMA targeting KFERQ motifs respectively, however the role of CMA in synapse development and differentiation is unknown. We focused in the development of excitatory synaptic development in cultured mouse hippocampal neurons. We analyzed the expression of the CMA receptor LAMP2A by western blot, immunofluorescence and confocal fluorescence microscopy at different stages of neuronal development. Additionally, a photoactivable fluorescent reporter of CMA (PA-mCherry-KFERQ) was used to determinate the presence of CMA active lysosomes in neuronal projections. In addition, a lentiviral shRNA mediated knockdown of LAMP2A was used to evaluate the effects of the loss of CMA activity in glutamatergic synapse development using immunofluorescence against pre- and post-synaptic proteins. We determined that LAMP2A expression increases in the period of synapse formation and reaches a peak during late stages of neuronal differentiation. LAMP2A positive lysosomes were found in close proximity to glutamatergic synapses, suggesting a potential local function of CMA active lysosomes near synapses. Additionally, PA-mCherry-KFERQ signal was compartmentalized in discrete domains in neurites in proximity with synaptic sites. shRNA mediated knockdown of LAMP2A induces a significant increase in the density of synaptic contacts. This was accompanied by changes in dendritic spine morphology and increases in the size of PSD95 protein clusters. In summary, our results indicate that CMA is present at neurites of hippocampal neurons and negatively modulates the morphological postsynaptic formation and maturation. Additional experiments with CMA gain and loss or function are in progress to determine if CMA activity alters functional synaptic development and maturation.

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Poster

198. Synaptogenesis and Activity-Dependent Development II

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Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 198.10/A48

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

Support: CIHR
NSERC

Title: Role of MEN1 gene in brain cell connectivity, synaptic plasticity and neurodegeneration

Authors: *S. BATOOL[1], J. M. ZAIDI[2], B. AKHTER[2], A. M. GETZ[1], N. I. SYED[3];
[1]Hotchkiss Brain Inst. and Neurosci., [2]Hotchkiss Brain Inst., [3]Cell Biol. and Anat., Univ. of Calgary, Calgary, AB, Canada

Abstract: The precise patterns of neuronal assembly during development determine all functional outputs of a nervous system, ranging from simple reflexes to learning, memory, cognition, etc. To understand how the brain functions normally, as well as how best to repair it after injury, disease or trauma, it is imperative that we first seek to define fundamental steps mediating this neuronal assembly. We made a novel finding that *MEN1* gene (tumour suppressor gene) plays role in the expression and synaptic maintenance of nicotinic acetylcholine receptors (nAChR), which are thought to regulate many aspects of synaptic plasticity. In this study using immunohistochemistry technique and confocal microscopy, we have shown that Menin, the product of *MEN1* gene, colocalizes at the presynaptic site with Synaptophysin and postsynaptically with PSD-95; a postsynaptic scaffolding molecule at excitatory neurons in mouse hippocampal cultures. Using *MEN1* shRNA lentivirus, we knocked down the expression of *MEN1* gene in primary hippocampal mice cultures and provide the first unequivocal evidence that *MEN1* knockdown leads to upregulation of Synaptophysin at presynaptic site and downregulation of PSD-95 at the postsynaptic site. We have also shown *MEN1* spatiotemporal localization in the mouse brain from E12.5, E15, E18 to an adult brain, using mouse brain slices. Our data demonstrate that *MEN1* gene may regulate many aspects of synaptic assembly underlying learning and memory circuits in the hippocampus.

Disclosures: **S. Batool:** A. Employment/Salary (full or part-time):; University of Calgary. **J.M. Zaidi:** None. **B. Akhter:** None. **A.M. Getz:** None. **N.I. Syed:** None.

Poster

198. Synaptogenesis and Activity-Dependent Development II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 198.11/A49

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

Support: Genetics Training Program 216381

Title: Early electrical synapses in the embryonic zebrafish spinal cord

Authors: *R. M. LUKOWICZ[1], A. ADKE[2], D. FRIEDMANN[3], A. C. MILLER[1];
[1]Inst. of Neurosci., Univ. of Oregon, Eugene, OR; [2]NIH, Washington, DC; [3]Dept. of Mol. and Cell Biol., UC Berkeley, Berkeley, CA

Abstract: The functionality of neural circuits are the result of the patterns and properties of the synaptic connections between neurons. Synapses are either chemical, where neurotransmitters are released and received, or electrical, where Connexin (Cx)-based gap junction channels (in vertebrates) mediate direct ionic and small molecule communication. Throughout the animal kingdom, electrical synapses couple neurons early in development; however, the molecular identity of the Cxs that form these first and early electrical synapses remains unknown. To address this question, we have utilized the embryonic zebrafish spinal cord and the first behavior elicited by the developing fish, spontaneous coiling, where the fish flexes its tail to the left and right in rhythmic patterns. This system provides an ideal model as the circuits are simple and accessible, and electrical synapses drive initial network function and behavior. The coiling circuit is initially composed of primary motoneurons (MN) and descending interneurons (dIN), which make local electrical synapses within a single segment, and later connect between segments to coordinate activity along the length of the spinal cord. To establish which of the 37 zebrafish Cxs may be forming early electrical synapses, we isolated coiling circuit neurons through FACs-sorting and identified Cxs enriched in these cells through RNA-sequencing. Utilizing CRISPR/Cas9, we knocked out target Cxs and screened for altered coiling behavior, which allowed us to isolate a previously uncharacterized Cx, Cx46.8, in which mutants display defects in the initiation, strength, and symmetry of coiling behavior over time. Whole embryo expression data indicates that Cx46.8 expression begins when coiling behavior starts (17hpf), and peaks when patterned neural activity is established (24hpf). This preliminary data suggests that Cx46.8 is contributing to the early electrical synapses that mediate coiling behavior in the fish. Future work will examine Cx46.8's role in coiling circuit function and maturation through identifying where it is expressed and forms gap junctions and examining how it contributes to coiling circuit function, which will provide insight into the role of early electrical synapses within the nervous system.

Disclosures: **R.M. Lukowicz:** None. **A.C. Miller:** None. **A. Adke:** None. **D. Friedmann:** None.

Poster

198. Synaptogenesis and Activity-Dependent Development II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 198.12/A50

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

Support: Brain Research Program (NRF-2015M3C7A1030964) funded by Korean National Research Foundation (NRF)

Title: Interaction of Ankycorbin and Tara regulates dendritic spine dynamics

Authors: S. KIM, Y. WOO, S.-J. NOH, Y. WON, E. CHO, *S. PARK;
POSTECH, Pohang, Korea, Republic of

Abstract: Dendritic spines undergo dynamic changes including formation, enlargement, shrinkage, and elimination during postnatal development, but become relatively stable as animals mature. Here, we report that an interaction of Ankycorbin and Tara modulates dendritic spine development and subsequent synaptic plasticity. Yeast-two-hybrid screening assay and co-immunoprecipitation assay revealed an interaction between Ankycorbin and Tara. Further studies of the interaction indicated that Tara increased the chemical stability of Ankycorbin protein. To identify the function of altered Ankycorbin protein level in dendritic spines, we removed Ankycorbin, Tara, or both from primary cultured hippocampal neurons. Regulation of Ankycorbin protein level in dendritic spines altered both number and size of dendritic spines, and their dynamics. These structural alterations coincided with changes of actin dynamics; this result suggests that Ankycorbin modulates dendritic spine dynamics by controlling actin dynamics. Our study suggests a novel Ankycorbin-dependent mechanism to regulate dendritic spines and will consequently contribute to increasing the understanding of dendritic spine dynamics.

Disclosures: **S. Kim:** None. **Y. Woo:** None. **S. Noh:** None. **Y. Won:** None. **E. Cho:** None. **S. Park:** None.

Poster

198. Synaptogenesis and Activity-Dependent Development II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 198.13/A51

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

Title: S-SCAM is essential for synapse formation and maintenance

Authors: *N. WITTENMAYER[1], J. S. VIOTTI[2], T. DRESBACH[2];
[1]Brandenburg Med. Sch., Brandenburg an der Havel, Germany; [2]Univ. Med. Ctr. Goettingen, Goettingen, Germany

Abstract: MAGI (membrane associated guanylate kinase inverted) proteins belong to the MAGUK-family (membrane associated guanylat kinase) of synaptic scaffolding proteins. They consist of three members: MAGI-1, MAGI-2 (also known as synaptic scaffolding molecule/S-SCAM) and MAGI-3. MAGI-2 or S-SCAM was originally characterized as a scaffold protein interacting with *N*-methyl-D-aspartate (NMDA) receptors at excitatory synapses. It is an essential synaptic scaffolding molecule for the GluA2-containing maintenance pool of AMPA receptors. Despite its function at matured synapses, little is known about the function of S-SCAM during early synapse formation. Using an RNAi-based knockdown approach in rat hippocampal neurons, we found that S-SCAM regulates synapse formation in general. Knockdown of all three S-SCAM isoforms during early synaptogenesis lead to a dramatic reduction of the number synapses on these cells and synaptic transmissions was impaired. Still formed synapses displayed misalignment of pre- and postsynaptic compartments and dendrite branching behavior was altered. In addition, the maintenance of matured synapses was reduced upon S-SCAM knockdown. These results suggest that despite its role at matured synapses, the postsynaptic scaffolding protein S-SCAM is crucial for proper synapse formation and maintenance.

Disclosures: N. Wittenmayer: None. **J.S. Viotti:** None. **T. Dresbach:** None.

Poster

198. Synaptogenesis and Activity-Dependent Development II

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

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MEXT KAKENHI 15H05772
MEXT KAKENHI 16K13107

Title: Activity-dependent secretion of Cbln1 from lysosomes in granule cell axons

Authors: *K. IBATA[1], M. KONO[2], S. NARUMI[1], J. MOTOHASHI[2], W. KAKEGAWA[2], K. KOHDA[1], M. YUZAKI[2];
[1]Physiol., St. Marianna Univ. Sch. of Med., Kanagawa, Japan; [2]Physiol., Keio Univ. Sch. of Med., Tokyo, Japan

Abstract: Cbln1 is a prototype of a new class of secreted synaptic organizers that potently mediate synapse formation and maintenance at the synaptic cleft. In the cerebellum, Cbln1 is produced by granule cells and plays crucial roles at parallel fiber (PF)-Purkinje cell synapses. However, how and when Cbln1 is released from neurons has remained unclear. In the present study, we reveal a new activity-dependent release mechanism for the synaptic organizer Cbln1 in granule cell axons. Cbln1 exocytosis, which can be triggered by physiological neuronal activities integrated over time, was insensitive to tetanus toxin, inhibited by dominant-negative forms of syntaxin4 and SNAP29, accompanied by release of a lysosomal enzyme cathepsin B, and inhibited by glycyl-L-phenylalanine 2-naphthylamide, which disrupt the lysosomal membrane, all indicative of a new form of lysosomal exocytosis from axons. Furthermore, overexpression of lysosomal sialidase Neu1 not only inhibited Cbln1 exocytosis *in vitro*, but also reduced PF axonal bouton formation *in vivo*. Therefore, we propose that activity-dependent release of Cbln1 from lysosomes onto the axonal surface, followed by lateral diffusion and capture at synapses, mediates the initial steps of PF synaptogenesis during development. As Cbln1 also mediates synaptogenesis in various regions of the forebrain, this release mechanism is likely widely utilized for activity-dependent synaptic modification by Cbln1 and its family proteins.

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Poster

198. Synaptogenesis and Activity-Dependent Development II

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Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 198.15/A53

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

Support: DFG

Title: Circuit growth is stabilized by negative feedback through anterograde trans-synaptic Jelly Belly - Anaplastic lymphoma kinase signaling

Authors: *P.-A. R. GÄRTIG[1], A. D. OSTROVSKY[1], L.-J. MANHART[1], T. KOVACEVIC[1], B. CHWALLA[2], M. LANDGRAF[2], S. CACHERO[3], J. F. EVERS[1];
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Abstract: The brain adapts to a changing environment or growing body size by structural growth and synaptic plasticity. These alterations are tightly coordinated between partner neurons to maintain circuit function. Positive feedback mechanisms synchronize synaptic growth. Negative feedback mechanisms that keep such reciprocal growth promotion in check, have so far remained elusive. Here, we analyze the interplay between neuronal growth dynamics and synaptogenesis by intra-vital imaging and quantification of endogenous synaptic protein accumulation at nanometer resolution in the motor system of *Drosophila*larvae. We identified trans-synaptic anterograde Jeb-Alk signaling as a negative feedback pathway that 1) acts locally to inhibit the addition of further postsynaptic dendritic specializations, and 2) elicits retrograde signaling that represses filopodia formation on presynaptic terminals, causing an indirect reduction in postsynaptic growth. Our findings demonstrate that Jeb-Alk signaling constitutes a negative feedback mechanism that mediates adaptive, but stable neuronal growth and synaptogenesis specifically during postembryonic circuit expansion.

Disclosures: P.R. Gärtig: None. **A.D. Ostrovsky:** None. **L. Manhart:** None. **T. Kovacevic:** None. **B. Chwalla:** None. **M. Landgraf:** None. **S. Cachero:** None. **J.F. Evers:** None.

Poster

198. Synaptogenesis and Activity-Dependent Development II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 198.16/A54

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

Support: NIH Grant NS097638

Title: Membrane palmitoylated proteins regulate synapse density and function in *C. elegans*

Authors: H. W. RICHBURG, B. MARKS, ***S. J. CHERRA, III**;
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Abstract: Synapse formation, function, and elimination are regulated by intercellular interactions. Cell adhesion molecules and secreted proteins modulate the formation of synapses. Downstream of the receptors and cell surface proteins, intracellular protein scaffolds, such as

membrane-associated guanylate kinases (MAGUKs), stabilize synapses and regulate synaptic plasticity. The MAGUK family of proteins is composed of several different subfamilies. Most notably, calcium/calmodulin-dependent serine protein kinase (CASK) and postsynaptic density 95 (PSD95) belong to two different MAGUK subfamilies and regulate synaptic function through presynaptic and postsynaptic mechanisms, respectively. Here, we investigated whether a third subfamily, the membrane palmitoylated proteins (MPPs), also regulates synaptic function using the *Caenorhabditis elegans* (*C. elegans*) locomotor circuit. Locomotion is regulated by acetylcholine induced muscle contraction and GABA induced muscle relaxation. To identify MPPs that regulate the locomotor circuit, we analyzed cholinergic function in animals that contained mutations in the MPP proteins: *magu-1*, *magu-2*, and *magu-3*. We investigated changes in postsynaptic or presynaptic function of the cholinergic neuromuscular junctions using a cholinergic receptor agonist or an inhibitor of acetylcholinesterase, respectively. We found that *magu-1* and *magu-2* mutant animals displayed changes in postsynaptic function, but *magu-3* mutants were indistinguishable from wild type animals. Interestingly, we found that only *magu-3* mutants were hypersensitive to the acetylcholinesterase inhibitor. To further understand the changes in synaptic function, we examined the cholinergic synapses in *magu-2* mutant animals. We observed that *magu-2* mutant animals contained more cholinergic neuromuscular junctions, suggesting that *magu-2* modulation of synapse number may underlie the hypersensitivity to the cholinergic receptor agonist. Based on these results, we propose that MPP proteins may modulate functions specific to pre- or post-synaptic compartments to regulate the locomotor circuit in *C. elegans*.

Disclosures: **H.W. Richburg:** None. **B. Marks:** None. **S.J. Cherra:** None.

Poster

198. Synaptogenesis and Activity-Dependent Development II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 198.17/A55

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

Support: NIH Grant NS106906

Title: A functional difference between NLGN4X and NLGN4Y

Authors: *R. HODGE[1], M. B. DALVA[2];
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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, less 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 on the Y chromosome with approximately 98% sequence homology to NLGN4X, often referred to as NLGN4Y. Yet the functional implications of expression of a male-specific vs. female-specific NLGN4 complement in humans are largely unexplored. We sought to test whether these proteins are found at synapses and have similar or distinct functions. We determined the synaptic localization of NLGN4 in the human brain and potential differences in function between NLGN4X and NLGN4Y *in vitro*. Using preparations from fresh human male and female temporal lobe, we have discovered that NLGN4X and NLGN4Y are expressed in the human brain and are located in the postsynaptic density. Similarly, we found that NLGN4X and NLGN4Y form heterodimers in synaptosomes isolated from fresh human male temporal lobe through proximity ligation assay. To assess a potential role in synaptogeneis, we used a heterologous co-culture assay and discovered that expression of NLGN4X or the co-expression of NLGN4X and NLGN4Y is sufficient for the formation of inhibitory, but not excitatory, synapses, while expression of NLGN4Y alone does not induce synapse formation. Furthermore, through site-directed mutatgenesis we identified a single amino acid residue in NLGN4X that is both necessary and sufficient for the formation of inhibitory synapses. Additionally, through immunofluorescent staining of male and female synaptosomes isolated from fresh human temporal lobe with pre- and postsynaptic markers we have shown that NLGN4 is found predominantly at inhibitory synaptosomes. Taken together, our studies shed light on a potential role for NLGN4 in inhibitory synapse formation in humans and has uncovered a functional difference between NLGN4X and NLGN4Y in synaptogenesis.

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

Poster

198. Synaptogenesis and Activity-Dependent Development II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 198.18/A56

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

Support: NS094171(R01)
NS076646 (R00)
Duke university faculty startup

Title: Ankyrin and CRMP regulate gap junction dynamics through Kinesin in C. elegance

Authors: *L. M. MENG[1], C.-H. CHEN[1], D. YAN[1,2];
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Abstract: The importance of gap junctions has been documented in many biological processes, yet the molecular mechanisms underlying gap junction dynamics remain unclear. To address this question *in vivo*, we use *C. elegans* PLM neurons as a model to study regulation of gap junctions. In a forward genetic screen, we isolated two mutants, *unc-44*/*ankyrin* and *unc-33*/CRMP (Collapsin Response Mediator Protein) altering distribution of gap junction plaques. Through time-lapse imaging experiments, we found *Ankyrin* and CRMP was required for gap junction dynamics and turnover. Genetic analysis showed that ankyrin acts upstream of CRMP in regulating gap junction dynamics through a kinesin (VAB-8). In summary, we first show a signal pathway involving ankyrin, CRMP and kinesin in regulating gap junction dynamics.

Disclosures: L.M. Meng: None. **C. Chen:** None. **D. Yan:** None.

Poster

198. Synaptogenesis and Activity-Dependent Development II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 198.19/A57

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

Support: NIH Grant R01NS078179
Start-up funds from Brown University and the Carney Institute for Brain Science
NIH Grant T32GM007133 (University of Wisconsin-Madison Predoctoral Training Program in Genetics)

Title: Uncovering the function of a non-canonical neuronal small GTPase

Authors: *K. MADHWANI[1], C. HOGAN[3], K. O'CONNOR-GILES[2,4];
[1]Neurosci. Grad. Training Program, [2]Neurosci., Brown Univ., Providence, RI; [3]Genet. Training Program, Univ. of Wisconsin- Madison, Madison, WI; [4]Carney Inst. for Brain Sci., Providence, RI

Abstract: *Drosophila melanogaster* has emerged as a powerful model to study synaptic genes conserved in humans. We have used spatial and temporal gene expression patterns from modENCODE to identify conserved, uncharacterized synaptic genes. Through genetic screening of these candidates, we identified candidate gene (CG) 8500 as a novel regulator of neuronal function. CG8500 encodes the sole *Drosophila* homolog of a family of non-canonical small GTPases known as DIRAS1-3 in mammals. DIRAS family members encode Ras-domain amino acid substitutions that alter sensitivity to inactivation by GAPs. DIRAS3, which is broadly

expressed, has been studied for its role in multiple cancers. In contrast, DIRAS1 and DIRAS2 are neuronally enriched and poorly understood despite emerging links to neurological disorders in genome-wide association studies. We have used CRISPR-Cas9 gene editing to generate genetic reagents for comprehensive study of CG8500/dDIRAS function *in vivo*. Loss of dDIRAS results in a significant decrease in adult locomotor activity. Additionally, dDIRAS regulates activity-dependent morphological plasticity. We will present our progress in understanding how this conserved, non-canonical small GTPase regulates synaptic function and plasticity.

Disclosures: K. Madhwani: None. **C. Hogan:** None. **K. O'Connor-Giles:** None.

Poster

198. Synaptogenesis and Activity-Dependent Development II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 198.20/A58

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

Support: NIH Grant R01 NS097161
NIH Grant R01 GM121931
NIH Grant R01 NS096509

Title: A new family of neural wiring receptors across bilaterians defined by phylogenetic, biochemical and structural evidence

Authors: *S. CHENG[1], Y. PARK[2], J. D. KURLETO[1], M. JEON[4], K. ZINN[4], J. W. THORNTON[3], E. OZKAN[1];
[1]Dept. of Biochem. and Mol. Biol., [2]Committee on Genetics, Genomics and Systems Biol., [3]Dept. of Human Genet., The Univ. of Chicago, Chicago, IL; [4]Div. of Biol. and Biol. Engin., Caltech, Pasadena, CA

Abstract: The evolution of complex nervous systems was accompanied by the expansion of numerous protein families, including cell-adhesion molecules mediating inter-cellular contacts between neurons. These proteins take part in neuronal wiring-related functions, such as axonal guidance and synapse targeting. We recently identified two families of the Ig superfamily (IgSF) in fruit flies that were shown to be expressed in combinatorial fashion in subsets of neurons in the brain and ventral nerve cord. Dprs and DIPs are involved in synaptic targeting and morphogenesis, retrograde signaling, and neuronal survival. Through structural biology we and others have established determinants of molecular specificity in Dpr-DIP interactions. Here, using evolutionary, biochemical, and structural techniques, we demonstrate that Dprs and DIPs are members of an ancient bilaterian family of receptors with neuronal wiring functions, hereby named as Wirins. We show that a single ancestral Wirin gene in the last common ancestor of Bilateria has given rise to the heterophilic Dprs and DIPs, and two other subfamilies in

protostomes, totaling 37 genes in *Drosophila*. The diversification of these subfamilies via gene duplication appears to have happened independently across protostome phyla. The ancestral Wirin evolved into the IgLON subfamily of neuronal receptors in vertebrates. We show that wild-type IgLONs can form homophilic and heterophilic interactions as predicted by their homologous relations to Dprs and DIPs, and their complexes can be broken by mutations designed using known Dpr and DIP structures. In nematodes, the Dpr and DIP orthologs, ZIG-8 and RIG-5, respectively, form strong heterophilic complexes and weak homophilic complexes. The crystal structures of the heterophilic and the two homophilic complexes reveal ancestral features common to other known Dpr-DIP complex structures. The multitude of relationships we have shown, supported by literature on the functions of various orthologs, provide strong links between evolution of cell adhesion molecules and the rise of the complex metazoan nervous system.

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Poster

198. Synaptogenesis and Activity-Dependent Development II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 198.21/A59

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

Title: Synthetic antigen binders for structural and functional studies of adhesion GPCRs

Authors: *S. P. KORDON, P. DUTKA, K. LEON, J. M. ADAMSKA, J. LI, A. SERETNY, A. A. KOSSIAKOFF, D. ARAC;
Univ. of Chicago, Chicago, IL

Abstract: G-protein coupled receptors (GPCRs) superfamily is the largest and most diverse family of cell membrane receptors in eukaryotes. They all share a common architecture, comprising of seven transmembrane helices, that inwardly transmit external signals by interactions between their N-terminal extracellular regions and different ligands including photon, ions, lipids, variety of small molecules and metabolites, peptides and proteins.
The adhesion GPCRs are the second largest subfamily of GPCRs, consisting of 33 members in humans. Adhesion GPCRs are characterized by an extended N-terminal extracellular region, possessing multiple adhesion domains capable of mediating cell-cell and cell-matrix interactions, and although most of them are orphan receptors with unknown functions, some members of this family have been shown to play important roles in a diverse range of biological functions such as myelination, angiogenesis and synapse formation. Adhesion GPCRs activity and mutations has been linked with a number of human conditions and neurological diseases.
Latrophilins (LPHNs) are members of the adhesion GPCRs family and are crucial in nervous

system development and function. Mutations of LPHNs has been associated with attention deficit hyperactivity disorder (ADHD) and numerous other psychiatric and neurological disorders. However, the molecular details of LPHNs and their mechanism of signaling has not been well-studied.
To gain insight into the LPHNs function, a potential solution is to use the synthetic antibodies (sABs) from phage display libraries. sABs are built from a Fab scaffold and biopanning strategies allow for the selection of region- and conformation-specific binders that can be used as structural chaperones for X-ray crystallography and cryo-electron microscopy, as well as specific biological probes to delineate signaling mechanisms. We have generated multiple high-affinity sABs to the LPHN protein variants. Here, we describe our initial characterization and validation of sABs selected for an extracellular domain of LPHN and its fragments, their binding specificity and ability of downstream signaling activation. From this work, we hope generation of high-affinity sABs will enable us to determine the structure of adhesion GPCRs in different conformational states, and ultimately aid our understanding of adhesion GPCRs functions.

Disclosures: **S.P. Kordon:** None. **P. Dutka:** None. **K. Leon:** None. **J.M. Adamska:** None. **J. Li:** None. **A. Seretny:** None. **A.A. Kossiakoff:** None. **D. Arac:** None.

Poster

198. Synaptogenesis and Activity-Dependent Development II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 198.22/A60

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

Support: NIH

Title: Identification of small molecule agonists and antagonists for the adhesion GPCR Latrophilin

Authors: *J. M. ADAMSKA, D. ARAC;
THE UNIVERSITY OF CHICAGO, CHICAGO, IL

Abstract: Latrophilins belong to the adhesion G-protein coupled receptor subfamily (aGPCRs), which are a novel and understudied GPCR family. These receptors are crucial in synapse formation and brain development. Mutations of Lphns are associated with attention deficit hyperactivity disorder (ADHD) and numerous cancers in humans. Despite Lphns critical role in neurobiology, no small agonist or antagonist are known. This study was aimed to identify small molecule activators and inhibitors for a Latrophilins. Cell-based high-throughput (HT) screening assays were used to identify small molecule agonist/antagonist of human Latrophilin Lphn (hLphn), which couple to Gα13 protein. HT assays were conducted at Northwestern High-Throughput Analysis Laboratory in Evanston. To detect Gα13-mediated signaling via SRE-

dependent transcription in cells, an SRE luciferase signaling assay was utilized. HEK293T cells transiently co-transfected with SRE luciferase reporter and hLphn were treated overnight with small molecule compounds from the MicroSource Custom Spectrum Collection. The same library of compounds was counter-screened using cells expressing the reporter and the constitutively-active Gα13 Q266L mutant. Results from 2700-compound Spectrum Collection suggest there are 70 hit compounds, which increase or decrease receptor activity. The top hit compounds were tested using Dual-Glo SRE-Luciferase Assay System in HEK293T cells. One of the tested compounds increases the Gα13 signaling, indicating that this particular small molecule can be a potential agonist of hLphn. These hit compounds, which could be possible agonists or antagonists, should be further characterized in future studies. These small molecules may be used as lead compounds for drug development of future treatments for diseases caused by malfunctioning aGPCRs.

Disclosures: J.M. Adamska: None. **D. Arac:** None.

Poster

198. Synaptogenesis and Activity-Dependent Development II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 198.23/A61

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

Support: NIH Grant

Title: Investigating the role of a putative *Drosophila* tRNA methyltransferase in neurons

Authors: *C. HOGAN[1], S. GRATZ[2], J. BRUCKNER[3], J. LENTINI[4], D. FU[4], K. O'CONNOR-GILES[2];
[1]Univ. of Wisconsin- Madison, Madison, WI; [2]Brown Univ., Providence, RI; [3]Univ. of Oregon, Eugene, OR; [4]Univ. of Rochester, Rochester, NY

Abstract: tRNAs are ubiquitous adaptor molecules that decode mRNAs through codon-anticodon base pairing and insertion of the appropriate amino acid into growing polypeptide chains. tRNAs undergo extensive posttranscriptional modifications that affect stability, efficiency, and fidelity. Although once thought to function as simple adaptor molecules, it is becoming apparent that tRNAs can function in the dynamic regulation of translation. Importantly, disruptions to the posttranscriptional processing of tRNAs have recently been linked to neurological disease. We identified a putative neuronal tRNA methyltransferase, TRM9L, as a novel regulator of synaptic growth through a genetic screen in *Drosophila*. TRM9L is one of two metazoan paralogs of a yeast enzyme, TRM9, that methylates uridines in the wobble position of the anticodon loop to modulate translational efficiency. Despite being characterized as a tumor suppressor in several human cancers, the biochemical function of TRM9L is unknown. We have

generated null alleles and confirmed that TRM9L mutants exhibit increased synaptic growth. Despite excess synaptic number, loss of TRM9L results in decreased synaptic transmission, suggesting an additional role in promoting neurotransmitter release. Finally, we find that TRM9L regulates sensitivity to reactive oxygen species in flies, consistent with its role in yeast stress response. We are combining genetic and biochemical studies to define the mechanism through which TRM9L carries out its diverse roles. These findings provide insight into how an expanded family of tRNA methyltransferases contributes to the regulation of neuronal function.

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Poster

198. Synaptogenesis and Activity-Dependent Development II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 198.24/A62

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

Support: Academy of Finland
Sigrid Juselius Foundation
Doctoral Program Brain and Mind at University of Helsinki

Title: GluK2 interacts with KCC2 to promote dendritic spine formation

Authors: *S. KESAF[1,2], S. KHIRUG[1], E. DINH[4], M. GARCIA[1], T. P. TAIRA[3], S. E. LAURI[2], C. RIVERA BAEZA[1,5];
[1]HiLIFE Neurosci. Ctr., [2]Mol. and Integrative Biosci. Res. Program, [3]Vet. Biosci., Univ. of Helsinki, Helsinki, Finland; [4]Developmental Biol., Aix-Marseille Univ., Marseille, France; [5]Inst. de Neurobiologie de la Méditerranée INMED UMR901, Marseille, France

Abstract: The interplay between glutamatergic and GABAergic transmission is crucial for the synaptic maturation during development. The impairments in the balance of neurotransmission may result in neurodevelopmental disorders, which are associated with the abnormal regulation of dendritic spines. The morphology of dendritic spines is regulated by the remodeling of actin cytoskeleton with a complex network of signaling molecules. Previous results have shown that KCl cotransporter, KCC2, has a morphogenic role in maturation of dendritic spines via the structural interaction with actin binding protein 4.1N. In addition, GluK2 kainate receptor subunit was shown to coexist in a functional complex with KCC2 to regulate its function and surface expression. In this study, we studied the role of GluK2-KCC2 interaction for the regulation of dendritic spine development. We found that shRNA-mediated GluK2 silencing in CA3 pyramidal neurons did not differ the density of total dendritic spines compared to controls *in vivo*, however the density and percentage of thin spines significantly increased, which is the

reminiscence of immature state. Consistent with this, GluK2 silenced CA3 neurons displayed a lower frequency of mEPSC. In control CA3 pyramidal neurons, KCC2 showed a strong perisomatic pattern of immunoreactivity, whereas a cytoplasmic pattern of immunoreactivity detected in GluK2 silenced CA3 neurons. Also, GluK2 silencing resulted in a smaller somato-dendritic gradient in $GABA_A$ equilibrium potential, reflecting the reduced efficacy of KCC2 function. In cultured rat hippocampal neurons, GluK2 silencing significantly reduced the density of dendritic spines, which was rescued by the overexpression of KCC2. We also studied the effect of GluK2 silencing on actin dynamics using fluorescence recovery after photobleaching (FRAP). FRAP data showed an increased stability of F-actin filaments in dendritic spines after GluK2 silencing as a result of increased phosphorylation ratio of actin depolymerizing factor cofilin. In conclusion, our results demonstrate that GluK2-KCC2 interaction developmentally regulates dendritic spine formation and its stability.

Disclosures: **S. Kesaf:** None. **S. Khirug:** None. **E. Dinh:** None. **M. Garcia:** None. **T.P. Taira:** None. **S.E. Lauri:** None. **C. Rivera Baeza:** None.

Poster

198. Synaptogenesis and Activity-Dependent Development II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 198.25/A63

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

Support: NIH: R01-GM120322

Title: Structural basis for teneurin function in circuit-wiring: A toxin motif at the synapse

Authors: *J. LI;
Univ. of Chicago, Chicago, IL

Abstract: Teneurins (TENs) are cell-surface adhesion proteins with critical roles in tissue development and axon guidance. Here we report the 3.1-Å electron cryo-microscopy structure of the human TEN2 extracellular region (ECR), revealing a striking similarity to bacterial Tc-toxins. The ECR includes a large β-barrel that partially encapsulates a C-terminal domain, which emerges to the solvent through an opening in the mid-barrel region. An immunoglobulin (Ig)-like domain seals the bottom of the barrel while a β-propeller is attached in a perpendicular orientation. We further show that an alternatively spliced region within the β-propeller acts as a switch to regulate trans-cellular adhesion of TEN2 to latrophilin (LPHN), a transmembrane receptor known to mediate critical functions in the central nervous system. One splice variant activates trans-cellular signaling in a LPHN-dependent manner, whereas the other induces inhibitory postsynaptic differentiation. These results highlight the unique structural organization of TENs giving rise to their multifarious functions.

Disclosures: J. Li: None.

Poster

198. Synaptogenesis and Activity-Dependent Development II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 198.26/A64

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

Title: Regulatory effects of non-coding RNAs in an iPSC-derived neuron model of human synapse development

Authors: *M. SOUTSCHEK, T. WÜST, L. VON ZIEGLER, S. BICKER, P.-L. GERMAIN, G. SCHRATT;
Dept. of Hlth. Sci. and Technol., ETH Zürich, Zurich, Switzerland

Abstract: Despite extensive research, there is up to date no final explanation for the unique cognitive abilities of the human species. Whereas most of the genetic, molecular and cellular components in mammalian brains are quite similar, human neurons seem to display an increased potential for synaptic plasticity. This might be due to a generally higher number of synapses or a prolonged maturation of these in comparison to other mammals and even primates. Non-coding RNAs and especially miRNAs play a significant role in synaptic development and plasticity. Furthermore, particularly the regulatory part of the genome distinguishes humans most from their closest relatives. Nevertheless, the contribution of non-coding RNA dependent mechanisms to human synapse development and plasticity is poorly understood. To study the role of non-coding RNAs in human synapse development, we established a defined human neuronal differentiation protocol without the need for the addition of animal glia cells on two different human induced pluripotent stem cell (iPSC) lines. With confocal microscopy, we show that the generated neurons constitute co-clustering of pre- and postsynaptic markers at Day 27 of differentiation as well as the formation of synaptic spines. Furthermore, the glia-free differentiation protocol allows us to perform small-RNA sequencing in combination with proteomics and long-RNA sequencing at defined developmental time points. Here, we present a first transcriptomic and proteomic characterization of the time course of human synapse development in iPSC-derived neuron cultures. These characterizations are essential for a more detailed subsequent functional analysis in the future. By manipulating the expression of specific candidate RNAs, we plan to investigate their role in synaptic maturation as well as effects on neuronal morphology and electrophysiological properties. Newly identified pathways can further be studied in the context of neuropsychiatric diseases using patient-derived iPSC lines.

Disclosures: M. Soutschek: None. **T. Wüst:** None. **L. von Ziegler:** None. **S. Bicker:** None. **P. Germain:** None. **G. Schratt:** None.

Poster

199. Genetic Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 199.01/A65

Topic: A.07. Developmental Disorders

Support: JSPS KAKENHI JP15H04645
JSPS KAKENHI JP18H02574
JSPS KAKENHI JP17K19488
JSPS KAKENHI JP17H03989
the JSPS Research Fellowships for Young Scientists JP17J00152
MEXT KAKENHI JP18H05416
AMED JP18dm0107122h0003

Title: ASD-associated *de novo POGZ* mutations disrupt cortical development

Authors: *K. MATSUMURA[1,2], K. SEIRIKI[1,2], M. NAGASE[3], S. AYABE[4], I. YAMADA[5], T. FURUSE[5], K. YAMAMOTO[1], K. KITAGAWA[1], M. BABA[1], A. KASAI[1], Y. AGO[1,6], A. H. TAKANO[1,7], N. SHINTANI[1], T. IGUCHI[8], M. SATO[8,9,10], S. YAMAGUCHI[11,12], M. TAMURA[5], S. WAKANA[5,13], A. YOSHIKI[4], A. M. WATABE[3], H. OKANO[14], K. TAKUMA[7,15], R. HASHIMOTO[16,17], H. HASHIMOTO[1,7,18,19], T. NAKAZAWA[1,15];
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Abstract: Autism spectrum disorder (ASD) is one of neurodevelopmental disorders characterized by reduced verbal communication abilities and social interactions, stereotyped repetitive behaviors and restricted interests. Recent genetic studies suggested that *de novo* mutations, genomic spontaneous mutations identified in an affected child, but not unaffected parents, play key roles in the etiology of ASD. In particular, genes with highly recurrent *de novo* possible loss-of-function mutations, which have been identified in multiple unrelated patients, are likely to contribute to the risk for ASD. Recently, we and other groups have identified that, among the high-confidence ASD risk genes, *POGZ* is one of the most recurrently mutated genes (at least 45 independent *de novo* non-synonymous mutations) in patients suffered from neurodevelopmental disorders, including ASD, suggesting that *de novo POGZ* mutations can be associated with the ASD pathogenesis. However, the biological significance of the *de novo* POGZ mutations and the role of POGZ itself in the brain remain unknown. In the present study, we examined the role of POGZ in the brain and functional characterization of *de novo POGZ* mutations.
We observed that *Pogz* expression was elevated in the mouse embryonic cerebral cortex during neurogenesis. We also found that *Pogz* silencing impaired the development of cortical neurons, which was rescued by overexpression of wild-type POGZ and *de novo* POGZ variants identified in unaffected controls but not by overexpression of ASD-associated *de novo* POGZ variants. Furthermore, the neuronal differentiation was disrupted in neural stem cells differentiated from iPS cells derived from an ASD patient carrying the *de novo POGZ* mutation. We also found that the *de novo POGZ* mutation resulted in disrupted cortical development and ASD-like features, including autistic abnormal behaviors, in mice. Our current results suggest that the ASD-related *de novo* POGZ variants may result in disrupted cortical development and ASD-like features.

Disclosures: K. Matsumura: None. **K. Seiriki:** None. **M. Nagase:** None. **S. Ayabe:** None. **I. Yamada:** None. **T. Furuse:** None. **K. Yamamoto:** None. **K. Kitagawa:** None. **M. Baba:** None. **A. Kasai:** None. **Y. Ago:** None. **A.H. Takano:** None. **N. Shintani:** None. **T. Iguchi:** None. **M. Sato:** None. **S. Yamaguchi:** None. **M. Tamura:** None. **S. Wakana:** None. **A. Yoshiki:** None. **A.M. Watabe:** None. **H. Okano:** None. **K. Takuma:** None. **R. Hashimoto:** None. **H. Hashimoto:** None. **T. Nakazawa:** None.

Poster

199. Genetic Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 199.02/A66

Topic: A.07. Developmental Disorders

Support: Research Council of Norway (grant#226971)

Title: Consensus principal component analysis identifies novel clusters of autism spectrum genes

Authors: D. L. PARKER[1], S. HASSANI[1], J. S. AXNÉR[1], C. E. REUTER[2], M. LU[1], E. HUO[1], *J. A. PINEDA[1], W. K. THOMPSON[3];
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Abstract: The heterogeneity of autism spectrum disorder (ASD) and its diagnostic overlap with other disorders complicate research and clinical practice yet also provide clues to underlying biological mechanisms. We examined *in silico* data for 638 genes, selected because they occur in ≥2 of 18 previously-identified ASD genetic modules. Consensus Principal Component Analysis (CPCA) was used to interrelate: (1) cellular function, (2) gene expression time and (3) association with comorbid disorders extracted from 6 gene databases for intellectual disability (ID), epilepsy, schizophrenia, neurodegeneration and dysmorphism. A cluster analysis based on principal component scores identified 7 clusters of genes (Cl1-Cl7) that comprise 3 general types (Fig. 1). Type 1 contains two clusters of regulatory genes, one involving chromatin remodeling and one MAPK signaling. Type 2 is characterized by genes with either early fetal gene expression or wide ranges of gene expression and multiple cellular functions. Of its 3 clusters, two are not generally associated with an annotated disorder and one is related to Intellectual Disability (ID) only. In contrast, Type 3 involves complex associations with comorbidities, including four distinct patterns of pleiotropy. We propose a model to account for unique or shared properties of clusters, thereby linking specific genetic modules or gene expression times to: (1) high- or low-functioning ASD, (2) ID or epilepsy accompanied by other comorbidities, (3) number of comorbidities per gene, (4) various transcription factors or regulatory mechanisms and (5) timing of gene expression potentially indicating sequential neural development. Notably, 83% of genes sharing the defining properties Cl2 (Type 1) and Cl7 (Type 3) are regulated by both CHD8 and FMRP. This relatively rare combination suggests a regulatory relationship between Cl2 (chromatin modification) and Cl7, which is enriched for FMRP regulation but also involves ID accompanied by multiple comorbidities. Taken together, our results describe 7 ASD subtypes that each link one gene set to a specific ASD comorbidity pattern. They illustrate the greater utility of comorbidity patterns, not individual disorders, in describing ASD heterogeneity.

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Poster

199. Genetic Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 199.03/A67

Topic: A.07. Developmental Disorders

Support: AMN Foundation
ERA-NET neuron AUTISYN and ADNPinMED
Gildor Chair
Elton Laboratory
Alicia Koplowitz Foundation
Drs. Ronith and Armand Stemmer

Title: ADNP, a brain protein with muscle and skin activities

Authors: O. KAPITANSKY, *I. GOZES;
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Abstract: Activity-Dependent Neuroprotective protein (ADNP) is an essential protein for brain formation and function, and is crucial for normal cognitive performance. Compared with *Adnp*$^{+/+}$ mice, *Adnp*-deficient mice survive, yet exhibit significant increase in phosphorylated tau, tangle-like structures, neurodegeneration, cognitive and social deficits, reversed by NAP, a snippet of 8 amino acids (NAPVSIPQ) derived from ADNP. Furthermore, ADNP was recently found to be frequently mutated in Autism Spectrum Disorders (ASDs) with associated cognitive, motor and sensory deficits, all mimicked by the *Adnp*-deficient mouse model, with NAP treatment reversing ADNP deficiencies. Specifically, our results indicated that *Adnp*-deficient mice have impaired neuromuscular activity, muscle tone and grip strength (hanging wire and grip strength tests), as well as gait deficits (CatWalk). Additional results suggested Neuromuscular Junction (NMJ) disruption and altered gene expression in the gastrocnemius muscle in *Adnp*-deficient mice, as compared with wildtype counterparts, all partially reversed by daily NAP administration (systemic/nasal). Human muscle gene expression data mining has revealed increased expression levels of *ADNP* and *ADNP2* in the vastus lateralis of old vs. young subjects, as well as altered expression of *ADNP* and *ADNP2* in the bicep brachii of elderly people, in a sex dependent manner. Furthermore, there was an inverse impact of prolonged exercise on the expression levels of both transcripts, with *ADNP* downregulated and *ADNP2* upregulated upon endurance training. An additional analysis found significant correlations between ADNP and 24 genes showing age-dependent changes in muscle transcript expression. Lastly, ADNP syndrome children carrying a specific causal heterozygous truncating mutation (p.Tyr719*) and *Adnp*-deficient mice both displayed skin abnormalities leading to a thinner epidermis and a delay in wound healing, partially ameliorated by NAP treatment. Together, these findings provide further evidence to the

phenotype of the ADNP syndrome, assisting in the characterization of this condition, as well as opening new avenues in research and future applicative therapeutics. Selected references: J Clin Invest. 2018 Nov 1;128(11):4956-4969; Sci Rep. 2019 Jan 24;9(1):736.

Disclosures: O. Kapitansky: None. **I. Gozes:** A. Employment/Salary (full or part-time):; Coronis Neurosciences.

Poster

199. Genetic Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 199.04/A68

Topic: A.07. Developmental Disorders

Support: SFARI Grant 314688, A. Gozzi
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2017 NARSAD Independent Investigator Grant, A. Gozzi
ERC, GA802371, A. Gozzi
Marie Skłodowska-Curie Global Fellowship, GA845065, M. Pagani

Title: A mechanistic link between mTOR-dependent deficient synaptic pruning and functional hyper-connectivity in autism

Authors: *M. PAGANI[1], A. BERTERO[1,2], A. DE FELICE[1], A. LOCARNO[3], I. MISEVICIUTE[3], S. TRAKOSHIS[4,5], C. CANELLA[1], K. SUPEKAR[6], V. MENON[6], A. GALBUSERA[1], R. TONINI[3], M. LOMBARDO[4,5], M. PASQUALETTI[1,2], A. GOZZI[1]; [1]Functional Neuroimaging Laboratory, Inst. Italiano di Tecnologia, Rovereto, Italy; [2]Dept. of Biology, Unit of Cell and Developmental Biol., Univ. of Pisa, Pisa, Italy; [3]Neuromodulation of Cortical and Subcortical Circuits Lab., Fondazione Inst. Italiano Di Tecnologia, Genova, Italy; [4]Dept. of Psychology, Univ. of Cyprus, Nicosia, Cyprus; [5]Lab. for Autism and Neurodevelopmental Disorders, Inst. Italiano di Tecnologia, Rovereto, Italy; [6]Stanford Univ., Palo Alto, CA

Abstract: Post-mortem examinations have revealed an excess of excitatory synapses in the brains of children with autism spectrum disorders (ASD). Recent investigations have linked this trait to hyper-activity of the mTOR pathway, resulting in synaptic pruning deficits (1). ASD is also characterized by alterations in brain functional connectivity as measured with resting state functional MRI (rsfMRI). These observations raise the question of whether and how mTOR-related deficient pruning affects rsfMRI dysconnectivity observed in ASD. Here we mapped dendritic synaptic density, rsfMRI connectivity (2) and social behavior in tuberous sclerosis 2 deficient ($Tsc2^{+/-}$) mice (n=20), a mouse line that mechanistically reconstitutes mTOR-dependent dendritic spine surplus observed in ASD (1). A separate cohort of animals (n=20), was subjected

to a daily treatment with the mTOR inhibitor rapamycin with the aim to rescue the synaptic surplus, and establish a link between synaptic traits and brain-wide connectivity. We observed increased spine density, socio-behavioral impairments, and long-range rsfMRI over-connectivity in basal ganglia and polymodal cortical areas of $Tsc2^{+/-}$ mice. Importantly, developmental treatment with rapamycin completely normalized synaptic density, rsfMRI hyper-connectivity and behavioral deficits in $Tsc2^{+/-}$ mice. These results implicate a causal link between mTOR-related pruning deficits and brain-wide rsfMRI hyper-connectivity. Given the prominent implication of mTOR pathway dysfunction for human ASD (1), we next hypothesized that a similar hyper-connected phenotype should be identifiable in clinical ASD populations. We therefore probed rsfMRI connectivity in pre-pubertal children from the ABIDE-I database (n=163 ASD, n=168 controls). This analysis revealed bilateral foci of increased long-range connectivity in the anterior insula of ASD children, a finding associated with a robust hyperconnection of this structure with the basal ganglia and polymodal cortices, recapitulating rsfMRI findings in Tsc2 mice. To mechanistically link this ASD imaging phenotype to TSC2- and mTOR-dependent signaling, we next asked whether genes within TSC2- or mTOR-networks are expressed in the human brain in a spatial pattern that is highly similar to the topology present in our imaging phenotype. This analysis revealed that TSC2/mTOR-network genes are indeed highly expressed in a similar topological pattern as the identified ASD hyper-connectivity phenotype. Taken together, our results support a mechanistic link between deficient synaptic pruning and rsfMRI over-connectivity in ASD. 1. Tang G et al, Neuron 2014 2. Bertero A et al, Brain 2018

Disclosures: **M. Pagani:** None. **A. Bertero:** None. **A. De Felice:** None. **A. Locarno:** None. **I. Miseviciute:** None. **S. Trakoshis:** None. **C. Canella:** None. **K. Supekar:** None. **V. Menon:** None. **A. Galbusera:** None. **R. Tonini:** None. **M. Lombardo:** None. **M. Pasqualetti:** None. **A. Gozzi:** None.

Poster

199. Genetic Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 199.05/A69

Topic: A.07. Developmental Disorders

Support: Telethon Foundation Grant GGP15110

Title: Altered L-type channel gating, action potential firing and inhibitory synaptic responses in hippocampal neurons of the autistic Timothy syndrome type-2 mouse

Authors: C. CALORIO[1], E. HIDISOGLU[4], D. GAVELLO[1], G. CHIANTIA[1], C. SALIO[2], M. SASSOE-POGNETTO[5], P. DEFILIPPI[3], E. TURCO[3], F. BALZAC[3], G. C. L. BETT[6], R. L. RASMUSSON[6], A. MARCANTONI[1], ***E. CARBONE**[1];

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Abstract: Tymothy syndrome (TS) is a multisystem disorder featuring cardiac arrhythmias, autism and adrenal gland dysfunction that originates from a *de novo* point mutation in the gene encoding the Cav1.2 (*CACNA1C*) L-type channel. Using the autistic TS2-neo mouse bearing the G406R point mutation associated with TS type-2 (Bader et al., 2011), we have recently shown that the mutation reduces the rate of inactivation and shifts leftward the activation and inactivation of L-type channels, causing marked increase of resting Ca^{2+} influx ('window' Ca^{2+} current) of adrenal mouse chromaffin cells (MCCs). The increased 'window current' causes marked reduction of Nav channel density, switches normal tonic firing to abnormal burst firing, reduces mitochondrial metabolism, induces cell swelling and decreases catecholamine release. Overnight incubation with nifedipine restores Nav channel density, normal MCC firing and quantity of catecholamine released (Calorio et al., 2019).
Here we report that in cultured hippocampal neurons (HNs; 3-10 days-in-vitro) of TS2-neo mutated mice, L-type calcium currents were less inactivated during pulses of 1 s to +10 mV. The voltage-dependence of activation and steady-state inactivation were both leftward shifted (-6 and -11 mV, respectively), as reported for MCCs. The shifts generated an increased resting window Ca^{2+} current. Immunolabeling of pyramidal and GABAergic neurons indicated a 15% loss of pyramidal neurons and a 8% loss of interneurons in TS2-neo cultures compared to WT. Current-clamp data analysis indicated the existence of two groups of neurons: a "slow-spiking" that was predominant in WT cultures and a "fast-spiking" that was predominant in TS2 cultures. The TS2-mutation increased the mean firing frequency of both groups but reduced to about 50% the number of HNs able to fire more than two APs, even under sustained depolarization.
In pharmacologically isolated GABAergic mono-synapses (Russo et al., 2018), the amplitude of electrically-induced IPSCs increased by 72% and the pair-pulse depression increased by 39% at 25 ms pulse separation in mutated HNs. In conclusion, the L-type channel gating changes induced by the G406R mutation of *CACNA1C* gene is most likely responsible for the increased resting window Ca^{2+} current, which results in reduced neuronal viability, altered excitability and a "gain of function" of GABAergic synaptic responses.

Disclosures: C. Calorio: None. **E. Hidisoglu:** None. **D. Gavello:** None. **G. Chiantia:** None. **C. Salio:** None. **M. Sassoe-Pognetto:** None. **P. Defilippi:** None. **E. Turco:** None. **F. Balzac:** None. **G.C.L. Bett:** None. **R.L. Rasmusson:** None. **A. Marcantoni:** None. **E. Carbone:** None.

Poster

199. Genetic Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 199.06/A70

Topic: A.07. Developmental Disorders

Support: AMN Foundation
ERA-NET neuron AUTISYN and ADNPinMED
Gildor Chair
Elton Laboratory
Alicia Koplowitz Foundation
Drs. Ronith and Armand Stemmer

Title: A novel genome edited mouse model for the ADNP syndrome

Authors: *G. KARMON, G. HACOHEN-KLEIMAN, S. SRAGOVICH, I. GOZES;
Tel Aviv Univ., Tel Aviv, Israel

Abstract: Autism Spectrum disorder (ASD) is a complex neurodevelopmental disease affecting ~1.5% of children worldwide with no known cure or clear etiology to date. ASD is highly heritable which implies shared genes and pathways. Activity-dependent neuroprotective protein (ADNP) was discovered by our laboratory as a protein essential for brain formation. In 2014, *de novo* truncating mutations in ADNP were identified as present in at least 0.17% of ASD cases, making ADNP one of the most frequent ASD associated genes. Patients exhibit intellectual disabilities, ASD and motor deficits, among their myriad of symptoms. With over 200 diagnosed patients and a projection of more than 20,000, it is of interest to further understand the *in vivo* function of ADNP, and its mechanism in relation to the ADNP syndrome and ASD. Using CRISPR-Cas9, we have established a heterozygous mouse model harboring the *Adnp* p.Tyr718* mutation (termed Adnp^{Tyr}) on a C57BL/6N background, homologous to the most prevalent human ADNP mutation, ADNP p.Tyr719*. While investigating this new model, interesting differences between the established Adnp haploinsufficient mouse model (termed Adnp$^{+/-}$, on ICR background) and the Adnp^{Tyr} model were observed. Furthermore, significant differences were also apparent between the different mouse backgrounds (ICR vs. a C57BL/6N). Both male and female Adnp^{Tyr} show no preference to mice over an empty cup in the social approach task, while the *wt* littermates show the normal tendency to explore the novel mouse. In contrast, only female Adnp$^{+/-}$ mice fail the social approach task. This finding is further augmented by the fact that, while the C57BLl/6N do not have intact odor discrimination, both genotypes of male ICR and female wt ICR show intact odor discrimination. Adnp$^{+/-}$ females cannot discriminate between different odors, offering a possible explanation for the social approach results in this model. In the long retention phase of the novel object recognition test (NOR), both sexes of the ICR background recall the familiar object, whereas the Adnp$^{+/-}$ mice do not. In the case of the C57BLl/6N strain, females of either genotype do not discriminate between the objects, while *wt* males do and Adnp^{Tyr} males do not. Our above findings show that the novel Adnp^{Tyr} mouse model mimics the human condition, as it presents autistic features and cognitive impairments in the social approach and NOR tasks. Therefore, with the advent of genetic manipulation techniques, such as CRISPR, the striking differences revealed here between sexes and background strains have strong implications on future ASD/ID mouse model generation in regards to background strain selection.

Disclosures: **G. Karmon:** None. **G. Hacohen-Kleiman:** None. **S. Sragovich:** None. **I. Gozes:** A. Employment/Salary (full or part-time):; Coronis Neurosciences.

Poster

199. Genetic Models for Autism Spectrum Disorders

Location: Hall A

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Program #/Poster #: 199.07/A71

Topic: A.07. Developmental Disorders

Support: Austrian Science Fund (F4410, F4402, P27809, W1101)
Jubiläumsfond of the Innsbruck Universities
Telethon Foundation (GGP15110)

Title: The autism-associated *de novo* A749G CACNA1D mutation induced a neurodevelopmental disease phenotype in mice

Authors: *N. J. ORTNER[1], N. HOFER[1], M. KHARITONOVA[1], E. PARADISO[2], P. TULUC[1], L. GUARINA[4], A. SAH[1], L. SCHWANKLER[1], N. STEFANOVA[3], F. FERRAGUTI[2], N. SINGEWALD[1], E. CARBONE[4], J. STRIESSNIG[1];
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Abstract: In the brain voltage-gated Cav1.3 L-type Ca^{2+} channels (LTCCs) are predominantly located postsynaptically and can shape cellular firing patterns, support pacemaker function and regulate Ca^{2+}-dependent gene expression. Germline *de novo* mutations in the channels' α1-subunit (encoded by the *CACNA1D* gene) have been found so far in eight patients with neurodevelopmental disease including autism spectrum disorder (ASD). Since heterozygous Cav1.3-deficiency does not cause a detectable brain pathology and the biophysical characterization of these Cav1.3 mutant channels in HEK293 cells showed gain-of-function features, we predict enhanced Cav1.3 mutant channel function *in vivo*. Therefore, already clinically available LTCC-inhibiting drugs, such as the dihydropyridine isradipine, might provide symptomatic benefits in affected individuals.
Here we report the successful generation and basic characterization of a novel mouse model (C57Bl/6N background) harboring the A749G *CACNA1D* mutation found in a patient with ASD and intellectual disability. The mutation does not affect the expression of Cav1.3 α1-subunit protein in the brain (Western blot) but induces the expected pathological gating changes of Cav1.3 currents in adrenal chromaffin cells isolated from heterozygous mutant mice. A delayed gain of body weight indicated a developmental delay in both male and female mutants. Behavioral analyses in adult male mice revealed a highly reproducible challenge-induced hyperlocomotion, reduced social interaction in the three chamber social test, a mild anxiety-like

phenotype in the light-dark test as well as reduced marble burying in a gene dose-dependent manner. No alterations of the gross brain morphology (Nissl-stained sections), number of dopaminergic midbrain neurons (unbiased stereology), cerebellar Purkinje cell density, striatal volume and cortical layering (primary motor/sensory and prefrontal cortex) have been observed between genotypes (adult males).
In summary, we showed - for the first time - the disease-causing nature of a human *CACNA1D* gain-of-function mutation evident as a neurodevelopmental disease phenotype including ASD-related behavioral alterations in mice expressing the ASD-associated A749G mutation. Since the phenotype was already present in the heterozygous state - reflecting the human situation - this mouse model represents an attractive tool to study the underlying Cav1.3-associated pathological mechanisms and to test potential therapeutic interventions with clinically approved LTCC-inhibiting drugs to improve ASD-related clinical symptoms in affected individuals.

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Poster

199. Genetic Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 199.08/A72

Topic: A.07. Developmental Disorders

Support: NIH T32 GM007067
Simons Foundation 571009
NIH/NIMH 5R01MH116999-02

Title: A massively parallel reporter assay to investigate the contribution of noncoding variation in autism spectrum disorder

Authors: *T. LAGUNAS, Jr[1], S. PLASSMEYER[2], J. D. DOUGHERTY[3];
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Abstract: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that affects ~1.7% of the population which leaves families and affected individuals with substantial lifetime costs. Current research has made significant investments in sequencing the genomes of ASD families as a diagnostic method and to further understand the genetic architecture of this complex disease. Whole Genome Sequencing (WGS) studies have revealed an enrichment of mutations in the untranslated regions (UTRs), which are noncoding regulatory regions, of ASD genomes. However, assessment of these variations poses a challenge since these regions do not follow the

triplet code and, even with prediction algorithms for RNA secondary structure or motif loss, these mutations must be defined experimentally. Furthermore, UTR sequences have been shown to have strong cell type dependency for their functionality; therefore, requiring assessment in appropriate cell types. To address these challenges, I have used a Massively Parallel Reporter Assay (MPRA) to functionally assay several hundred mutations in parallel. MPRAs are a novel molecular genetic tool for assaying hundreds to thousands of predefined sequences for functional effects in a high-throughput manner. From a completed assay that looked at 650 3' UTR mutations from ASD genomes, I have reported several candidates 3' UTR variations that appear to have functional effects on mRNA stability - indicating that some of these mutations may contribute to disease. Implementing post-transcriptional MPRAs, and cell type specific translational profiling, I aim to 1) extend these findings by testing their impact in disease relevant tissues such as the mouse brain and human cells, 2) define the molecular mechanisms that alter protein levels for specific UTR mutations, 3) determine the mechanism of action for a putative ASD risk variant to potentially implicate a new gene in this disease. This work will justify the significant investments in sequencing patients with ASD by reporting on the burden of noncoding disease mutations and contribute to our understanding of ASD genetic architecture and UTRs.

Disclosures: **T. Lagunas:** None. **S. Plassmeyer:** None. **J.D. Dougherty:** None.

Poster

199. Genetic Models for Autism Spectrum Disorders

Location: Hall A

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Program #/Poster #: 199.09/A73

Topic: A.07. Developmental Disorders

Support: 2016 NARSAD Young Investigator Grant from the Brain & Behavior Research Foundation
FOXG1 MH109648
ACE P50 MH115716
Psychencode U01 MH103365
Chromatin Regulation during brain development R56 MH114911

Title: Deconvoluting cell type composition of brain organoids derived from autistic patients and controls by single-cell RNA-sequencing

Authors: *J. MARIANI[1], F. WU[1], A. AMIRI[1], C. K. NGUYEN[1], A. JOURDON[1], A. ABYZOV[3], F. M. VACCARINO[2];
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Abstract: In our previous study using iPSC-derived telencephalic organoids from families with idiopathic autism and macrocephaly we identified an unbalanced overproduction of inhibitory neuron precursors in ASD organoids as compared to organoids derived from their unaffected family members. Consistently, bulk RNA-sequencing and gene co-expression network analyses revealed an upregulated transcript module enriched in genes that govern telencephalic neuron differentiation, including several transcription factors regulating the acquisition of glutamatergic/GABAergic cell fate (Mariani et al., 2015).We have deconvoluted the cell type gene expression of ASD- versus control-derived brain organoids by single-cell RNA-sequencing (scRNA-seq) in order to evaluate if this increase in expression of GABAergic gene products is due to an increased number of cells of GABAergic lineages or to a change in gene expression in GABAergic cells, or both.Characterization of organoid cell groups based on single cell transcriptome revealed several subtypes of radial glia (RG) revealing an evolution from ventricular RG at terminal differentiation (TD) 30 to outer RG at TD 60, as well as excitatory and inhibitory neuronal cell subgroups. Every cell cluster in organoids matches to one or a few of those found in human late embryonic/fetal telencephalon, suggesting that our organoid system largely recapitulates the development of human developing telencephalon.Moreover, by mixing together iPSCs from ASD-patients and controls we are dissecting out cell-autonomous and non-cell-autonomous effects driving the differences in neural subtype composition and gene expression between ASD- and control-derived organoids.

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Poster

199. Genetic Models for Autism Spectrum Disorders

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Program #/Poster #: 199.10/A74

Topic: A.07. Developmental Disorders

Title: Investigating the role of Janus kinase and microtubule interacting protein 1 (JAKMIP1) as an autism candidate gene using CRISPR-Cas9 genome editing technique

Authors: *N. TAKAMURA[1,2], J. GANDAWIJAYA[2], R. A. BAMFORD[2], C. DAVIS[2], J. K. CHILTON[2], A. OGURO-ANDO[2];
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Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder estimated to affect 1% of the global population, is typically characterized by difficulties of social interaction and communication, and repetitive/restricted behaviour. Previous studies compared syndromic forms of ASD with either Fragile X syndrome (FXS) or 15q duplication syndrome comorbidities and

identified *Janus Kinase and Microtubule interacting protein 1* (*JAKMIP1*) as a potential molecular link between these two forms of ASD. In recent research, JAKMIP1 was shown to regulate neuronal translation as a component of the Fragile X Mental Retardation Protein (FMRP)-associated complex and JAKMIP1 KO mice demonstrated several core ASD behaviors including repetitive behaviors and social deficits. However, it is still not clear how JAKMIP1 dysregulation leads to the neuronal dysfunctions and results in syndromic and idiopathic ASD. Interestingly, JAKMIP1 interacts with Janus Kinase (JAK) family members such as JAK1 and TYK2. In general, JAK-STAT regulates cytokine signaling in the immune system and has various roles in normal brain functions, including synaptic plasticity and modulation of neuroreceptor functions. Therefore, we aimed at identifying roles of JAKMIP1 in JAK-STAT signaling in neurons and utilized the CRISPR-Cas9 system to knockout *JAKMIP1* in human neuroblastoma cell lines.
To disrupt the JAKMIP1 gene, we tried two approaches; transfecting Cas9 with single gRNA or with double gRNAs. Both approaches successfully induced mutations at targeted sites. We are currently planning to perform RNA sequencing using this model and the data will support to identify novel cellular pathway candidates for pharmacological modulation in ASD.

Disclosures: N. Takamura: None. **J. Gandawijaya:** None. **R.A. Bamford:** None. **C. Davis:** None. **J.K. Chilton:** None. **A. Oguro-Ando:** None.

Poster

199. Genetic Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 199.11/A75

Topic: A.07. Developmental Disorders

Support: Hussman Foundation #15005

Title: GABAergic interneuron defects in mice lacking autism-associated gene Slit3

Authors: K. MENZEL, S. EDWARDS, ***C. P. PLACHEZ**;
Hussman Inst. for Autism, Baltimore, MD

Abstract: **Background:** Neuronal connectivity defects have been reported in individuals with Autism Spectrum Disorder (ASD) and lead to alterations in brain function and multisensory integration. In particular, SLIT3, an axon guidance molecule, attracted our attention as it is expressed during brain development, has mutations in individuals with autism, and may be associated with neurological conditions such as major depressive disorder and schizophrenia. We have found that mice lacking autism-associated gene Slit3 display ASD-like behaviors and have a reduced number of GABAergic interneurons in key brain areas associated with ASD. Taken together, these findings suggest that Slit3 may be an attractive candidate to analyze altered

neuronal connectivity in ASD. **Objectives:** We hypothesize that loss of Slit3 gene leads to abnormal GABAergic cell distribution, affecting neuronal connectivity and Excitation/Inhibition (E/I) balance. Therefore in this study we aim to investigate GABAergic interneuron neuronal connectivity in Slit3 mutant mice. **Methods:** Neuronal connectivity was analyzed from GABAergic interneuron cell cultures using a live-cell analysis system. This methods allows for reliable tracking of cell health and morphology, neurite analysis and neuronal function. **Results:** GABAergic interneurons were collected at embryonic day E14.5 and plated for neuronal connectivity analysis. Using the Incucyte live-cell analysis system we were able to analyze GABAergic interneuron cell properties and analyze dynamic changes and cell interactions in both Slit3 mutant mice and control conditions. Our preliminary results show that GABAergic interneurons collected from mice lacking autism-associated gene Slit3 display differences in cell health and morphology. Neurite analysis assays also show that GABAergic interneurons lacking the Slit3 gene exhibit differences in both neurite formation and neurite outgrowth compared to control GABAergic interneurons. **Conclusions:** Interneurons are known to synchronize neuronal activity and this synchronization is essential for cortical network function. Our results show that loss of autism-associated gene Slit3 affects GABAergic interneuron mediated connectivity. These findings reveal the importance of Slit3, an axon guidance molecule, in the formation of GABAergic neuronal networks and provide insight into the molecular pathways that may lead to altered neuronal connectivity in ASD.

Disclosures: K. Menzel: None. **S. Edwards:** None. **C.P. Plachez:** None.

Poster

199. Genetic Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 199.12/A76

Topic: A.07. Developmental Disorders

Support: NIH Grant NS100954
NIH Grant NS099188

Title: Deletion of the Kcna1 epilepsy gene modifies autistic-like behaviors

Authors: I. JAGADEESWARAN[1,2], A. PRUITT[2], N. M. GAUTIER[2], K. CRANE[2], ***E. GLASSCOCK**[1,2];
[1]Southern Methodist Univ., Dallas, TX; [2]Louisiana State Univ. Hlth. Sci. Ctr., Shreveport, LA

Abstract: Epilepsy and autism are highly comorbid, co-occurring in up to 30% of patients, which suggests shared genetic etiology, underlying pathophysiology, and/or neurodevelopmental abnormalities; however, the exact nature of this relationship remains unclear. In this study, the goal was to investigate how mutations in two ion channel genes, one associated with epilepsy

(*Kcna1*) and one with autism (*Scn2a*), interact to modify genotype-phenotype relationships in the context of epilepsy-autism comorbidity. Previous work has shown that partial deletion of *Scn2a* (*Scn2a*$^{+/-}$), which encodes Nav1.2 subunits, acts as a protective genetic modifier of epilepsy phenotypes in *Kcna1*-null (*Kcna1*$^{-/-}$) mice, which lack Kv1.1 subunits. Therefore, in this work the reciprocal hypothesis was tested: that deletion of the *Kcna1* epilepsy gene can act as a beneficial genetic modifier of autistic-like features in *Scn2a*$^{+/-}$ mice. To examine this, mice with various combinations of *Kcna1* and *Scn2a* knockout alleles (n=7-12/genotype) were evaluated for the presence of abnormal repetitive (marble burying, self-grooming, and nestlet shredding) and social (sociability and social novelty) behaviors, which are associated with autistic-like deficits. *Kcna1*$^{-/-}$ mice exhibited significant decreases in all repetitive behaviors (P<0.01), but their social interactions were unaltered compared to wildtype (WT). *Scn2a*$^{+/-}$ mice displayed increased self-grooming (P=0.009), decreased marble burying (P=0.0007), and altered social novelty (P=0.003) compared to WT. Complete genetic ablation of *Kcna1* in *Scn2a*$^{+/-}$ mice (i.e., *Scn2a*$^{+/-}$*; Kcna1*$^{-/-}$) led to decreased repetitive behaviors similar to single mutant *Kcna1*$^{-/-}$ mice. In contrast, however, partial deletion of Kv1.1 potassium channels in *Scn2a*$^{+/-}$ mice (i.e., *Scn2a*$^{+/-}$*; Kcna1*$^{+/-}$) had a selective therapeutic effect, normalizing self-grooming repetitive behavior (P=0.02) and social novelty (P=0.045) to levels indistinguishable from WT while not significantly modifying other autistic-like behaviors. Our results show that not only is *Scn2a*$^{+/-}$ beneficial in the context of epilepsy due to *Kcna1* mutation but that *Kcna1*$^{+/-}$ is also beneficial in the context of autistic-like features due to *Scn2a* mutation. Thus, epilepsy- and autism-associated ion channel mutations can act reciprocally as mutually beneficial genetic modifiers to reduce disease severity.

Disclosures: I. Jagadeeswaran: None. **A. Pruitt:** None. **N.M. Gautier:** None. **K. Crane:** None. **E. Glasscock:** None.

Poster

199. Genetic Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 199.13/A77

Topic: A.07. Developmental Disorders

Title: Altered neural progenitor proliferation associated with copy number variants in psychiatric disorders

Authors: *T. BRICKLER, J. LI, A. BANUELOS, K. MARJON, J. BIAN, S. CHETTY; Stanford Univ., Stanford, CA

Abstract: Copy number variations (CNVs) of certain chromosomal regions are closely associated with neurodevelopmental and neuropsychiatric disorders such as autism spectrum disorder (ASD) and schizophrenia. Recent evidence suggests that alterations in neural progenitor

cell (NPC) proliferation associated with particular CNVs may underlie the cause of abnormal brain development. However, we still have limited knowledge of the cellular and molecular profiles of NPCs during neurodevelopment that adds to the complexity of these diseases, which can affect multiple cell types in different regions of the brain. In order to investigate the cellular and molecular mechanisms underlying neuropsychiatric disorders, we have used NPCs and neurons derived from human induced pluripotent stem cells (hiPSCs). Here, we generated NPCs and neurons from patients diagnosed with a psychiatric disorder and particular CNVs to better model these disorders and identify molecular targets for intervention. We have found that proliferation rates of NPCs can be closely tied to specific CNVs, allowing us to group patients by particular disease phenotypes to help gain insight into the disruption of cellular and molecular pathways during development. Interestingly, these differences in NPC proliferation pathways mechanistically converge with neuroimmune mechanisms. To identify how these signaling roles play in cell survival and clearance, we have co-cultured our derived NPCs and neurons with immune-derived cells and assessed differences in cellular elimination. Many forms of psychiatric disorders such as ASD and schizophrenia are associated with abnormal synapse connections which could allude to improper elimination of NPCs that lay the groundwork for the brain architecture. Our study will be the first to systematically investigate novel neuroimmune-related mechanism(s) tied to ASD and schizophrenia as well as correlate a clinical phenotype to a cellular phenotype.

Disclosures: T. Brickler: None. **J. Li:** None. **A. Banuelos:** None. **K. Marjon:** None. **J. Bian:** None. **S. Chetty:** None.

Poster

199. Genetic Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 199.14/A78

Topic: A.07. Developmental Disorders

Support: Yerkes National Primate Research Center Pilot Grant NIH/OD P51 OD011132-56
Yerkes National Primate Research Center Base Grant (OD P51OD011132)
University Research Committee (URC) 2019 Interdisciplinary Award, Emory University

Title: Identifying genetic variants associated with social phenotypes of relevance for autism spectrum disorder (ASD) in juvenile rhesus macaques

Authors: *Z. A. KOVACS-BALINT[1], C. GUNTER[2,6,3], A. R. HARRIS[7], M. RAVEENDRAN[7], V. MICHOPOULOS[1,4], J. BACHEVALIER[1,5], J. RAPER[1,2], M. SANCHEZ[1,4], J. ROGERS[7];
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Psychiatry and Behavioral Sci., [5]Dept. of Psychology, Emory Univ., Atlanta, GA; [6]Marcus Autism Ctr., Children's Healthcare of Atlanta, Atlanta, GA; [7]Human Genome Sequencing Ctr., Baylor Col. of Med., Houston, TX

Abstract: Autism spectrum disorder (ASD) is a developmental disorder with high heritability and equal contributions of genetics and environment to the overall risk. Due to limits on experiments in human infants, we seek a translational nonhuman primate (NHP) model. The rhesus monkey, with complex social behaviors, brain anatomy and ontogeny closely resembling human, provides a critical alternative to study the origins of atypical social behaviors. The goal of this study was to identify genetic variants associated with social phenotypes of relevance for ASD in juvenile macaques. To select social phenotypes, we validated the Social Responsiveness Scale (SRS), used in ASD diagnosis and research in humans and previously adapted to adult macaques, in 209 juvenile macaques living in complex social groups at the Yerkes National Primate Research Center. We reduced the macaque SRS (mSRS) to 14 items, established the underlying factor structure using exploratory factor analysis (EFA), and validated it for juvenile macaques (jmSRS). We also collected two hours of behavioral observations per animal using a well-established ethogram for this species. Separately, we performed whole exome sequencing (WES) on DNA samples using the Rhexome v2 capture reagent and the Illumina NovaSeq system. Reads were aligned to the rhesus Mmul_8.0.1 reference genome. We called genomic variants using the GATK package and performed preliminary genetic associations tests to identify genetic variants that influence the phenotypes. We used CADD scores to predict the functional impact of variants of interest in a list of 87 candidate genes of interest for ASD.Analysis of the jmSRS revealed similarities to the human SRS, confirming its construct validity in juvenile macaques. Using the jmSRS and behavioral observations, we identified species- and age-atypical behaviors as well as extreme social phenotypes. Our exploratory WES yielded 1,350 single nucleotide variants (SNVs) and 95 indels in the selected gene list. Analyses also revealed two genomic variants - CHD8 and KDM6B - in our macaque colony, with significant associations to atypical social behaviors (based on the jmSRS and the behavioral observations).Our findings show a methodological advancement in the development of a NHP animal model with high translational value for ASD. We are further analyzing rhesus variants (alone and in combinations) linked to atypical social behaviors.

Disclosures: Z.A. Kovacs-Balint: None. **C. Gunter:** None. **A.R. Harris:** None. **M. Raveendran:** None. **V. Michopoulos:** None. **J. Bachevalier:** None. **J. Raper:** None. **M. Sanchez:** None. **J. Rogers:** None.

Poster

199. Genetic Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 199.15/A79

Topic: A.07. Developmental Disorders

Support: KHIDI, HI18C1077

Title: Behavioral and cellular deficit in neurodevelopment of Chloride channel-4 knock out mice

Authors: *Y. KIM[1], S. JEON[2], J. HAN[3];
[1]Natl. Ctr. For Mental Hlth., Seoul, Korea, Republic of; [2]Dongguk Univ. Res. Inst., Gyenggi-do, Korea, Republic of; [3]Grad. Sch. of Med. Sci. and Engin., KAIST, Daejeon, Korea, Republic of

Abstract: Chloride channel enable influx of chloride ion causing hyperpolarization of the membrane. In this study we investigated the neurodevelopment of chloride channel 4 knockout mice. We conducted open field test, Y maze test, marble burying test, and social interaction test in the wildtype and chloride channel 4 knock out mice. We performed Golgi staining in both wildtype and knockout mouse brain and compared the neuronal cytoarchitecture. Primary cell culture was performed of the fetal brain tissue at embryonic day 14 to investigate the neuronal differentiation process of the chloride channel 4 knock out mice compared to wildtype. We observed that total arm entry was increased in knockout mice compared to wildtype mice in Y-maze test. Total time spent in open arm was also increased in chloride channel 4 knock out mice compared to wildtype. Knockout mice spent less time exploring the novel mice compared to wildtype mice in social interaction assessment. We also observed cytoarchitectural abnormalities of the brain using Golgi staining in chloride channel 4 knock out mice compared to wildtype mice. Finally, we found that chloride channel knockout mice showed slower neuronal differentiation compared to wildtype mice. In conclusion, chloride channel knockout mice show both behavioral and cellular deficit during neurodevelopment compared to wildtype mice.

Disclosures: Y. Kim: None. **S. Jeon:** None. **J. Han:** None.

Poster

199. Genetic Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 199.16/A80

Topic: A.07. Developmental Disorders

Support: This work was supported by a generous gift from Eric and Wendy Schmidt by recommendation of the Schmidt Futures Program
David Haussler is a Howard Hughes Medical Institute Investigator.
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Title: Identification of novel alleles of a human specific neocortical gene, NOTCH2NL associated with neurodevelopmental disorders

Authors: *N. R. HEYER[1,4], C. BOSWORTH[1,2], G. MANTALAS[1,3], D. HAUSSLER[1,2,5], S. SALAMA[1,2,5];
[1]Genomics Inst., [2]Biomolecular Engin. and Bioinformatics Dept., [3]Molecular, Cell. and Developmental Biol. Dept., Univ. Of California, Santa Cruz, Santa Cruz, CA; [4]California State University, Monterey Bay, Seaside, CA; [5]Howard Hughes Med. Inst., San Francisco, CA

Abstract: The three NOTCH2NL genes, NOTCH2NLA, NOTCH2NLB, and NOTCH2NLC are implicated in 1q21.1 distal deletion/duplication syndromes, which can result in neurodevelopmental disorders (NDs) such as Autism, ADHD, Schizophrenia, Microcephaly, and Macrocephaly. Previous work demonstrated that NOTCH2NLA, NOTCH2NLB, and NOTCH2NLC are located within the typical deletion and duplication region, and functional analysis suggested a role for NOTCH2NL in regulating neural stem cell expansion in the developing cerebral cortex.[1,2] We aim to understand the differing relationships of specific NOTCH2NL alleles with NDs. By understanding their function, we open the possibility for a future clinical diagnostic model to predict the likelihood of NDs resulting from specific NOTCH2NL allele combinations. To do this, we first developed an analysis pipeline to determine the NOTCH2NLA, NOTCH2NLB, and NOTCH2NLC alleles present in individual samples, as well as the related genes NOTCH2NLR and NOTCH2. This pipeline utilizes targeted linked read sequencing of high molecular weight genomic DNA, that allows us to obtain the high-depth and base-accurate reads needed to assemble genes in the presence of short tandem repeats.[1] Reads are grouped based on self-similarity and classified into groupings corresponding to each gene in the family. Groupings are then translated in silico into the corresponding amino acid sequence, allowing for the identification of protein variants. We used this pipeline to analyze the alleles of the NOTCH2NL gene family in a genetically diverse pool of individuals from the Thousand Genome Project[3], sequenced by Genome in a Bottle[4] (n = 7) and in our lab (n = 13). We discovered eight novel NOTCH2NL alleles, including a well-supported frameshift mutation in NOTCH2NLC, the result of a deletion of a single base pair in the last exon, in the region coding for novel sequence relative to the parent NOTCH2 gene. The analysis and discovery of these new alleles are promising, as this is the first step in determining the relative neurodevelopmental function of the different loci and alleles. We plan to take advantage of large genome sequencing data sets such as those generated by the Autism Genetics Resource and the Simons Foundation Autism Research Initiative to correlate NOTCH2NL alleles with NDs. We foresee this work eventually allowing for the development of a clinical model that could allow for earlier detection of these disorders and, in turn, could help to create better patient outcomes.
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3. Auton A et al. (2015) Nature. 526:68.
4. Zook JM et al. (2016) Scientific Data. 3:160025.

Disclosures: N.R. Heyer: None. **C. Bosworth:** None. **G. Mantalas:** None. **D. Haussler:** None. **S. Salama:** None.

Poster

199. Genetic Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 199.17/A81

Topic: A.07. Developmental Disorders

Support: R03 MH 104851
R03 AG 056956

Title: Behavioral and neuroanatomical alterations in mouse models of autism spectrum disorder

Authors: *T. GANDHI, C. LEE;
Comparative Biomed. Sci., Louisiana State Univ., Baton Rouge, LA

Abstract: Autism spectrum disorder (ASD) is comprised of a group of neurodevelopmental disorders characterized by deficits in social interaction and communication along with restricted interests and repetitive behaviors. Hence, the autistic phenotype exhibits diverse behavioral impairments with underlying etiologies that remains elusive.
Imbalance in neuronal excitation and inhibition (E:I) have been implicated in aberrant neuronal connectivity contributing to the complex behavioral symptoms associated with the disorder. In this study, we examine two genetic mouse models of ASD: one harboring a knockout of the contactin associated protein-like 2 gene (*CNTNAP2*$^{-/-}$), and the other containing a homologous regional deletion of human chromosome 16p11.2.
Using these genetic mouse models, we investigated the abnormal behavioral repertoire and underlying neuroanatomical alterations in cortical areas that might underlie asynchronous brain rhythmicity.
One of the important neuropathological features in these genetic mouse models of ASDs is the abnormal developmental migration of neurons destined for superficial cortical layers. In our study, the laminar organization of the cortical areas was analyzed by labeling with CUX-1, which is a marker for neurons normally localized to the superficial cortical layers (II-IV). Additionally, retrograde tract tracing was employed in order to map cortical projection patterns. Furthermore, analysis of core behavioral domains incorporated ultrasonic vocalization recordings in pups, grooming and social interaction tests in adult mutant and control groups. By quantifying the laminar distribution of CUX-1 positive cells in the cortical areas of these genetic models of ASDs, our findings show increased number of CUX-1 positive cells mis-localized to the lower layers (V and VI) of these cortical areas as compared to the control animals. Furthermore, our results indicate reduced ultrasonic vocalizations in mutant mouse pups as compared to controls. Also, the mutant group exhibited a decrease in sociability and increased grooming in comparison to controls. These results suggest that neuroanatomical alterations in cortical areas could account

for some of core autistic
behaviors.

Disclosures: **T. Gandhi:** None. **C. Lee:** None.

Poster

199. Genetic Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 199.18/A82

Topic: A.07. Developmental Disorders

Support: PACS1 Syndrome Research Foundation

Title: Exploring an autism-linked mutation in the PACS1 gene using stem cells and cerebral organoids

Authors: *L. E. RYLAARSDAM[1], A. D. GUEMEZ-GAMBOA[2];
[2]Physiol., [1]Northwestern Univ., Chicago, IL

Abstract: Schuurs-Hoeijmakers Syndrome (SHMS) is a neurodevelopmental disorder characterized by global developmental delays and similar craniofacial abnormalities, resulting from a single mutation in the phosphofurin acidic cluster sorting protein-1 (PACS1). Due to the relatively recent discovery of the disease, very little is known about its function in the context of the developing nervous system, and few therapies are available to patients. Here we introduce the characteristic R203W mutation into stem cells using CRISPR. We use this modified cell line in addition to patient-derived stem cells to assess impairment of physiological properties in neuronal cell types. In addition to two-dimensional assays, we also use cerebral organoids as a novel platform to study the disease mechanism. This is the first time the PACS1 mutation found in SHMS has been extensively studied in neuronal cell types, which will be critical for developing effective therapies in patients.

Disclosures: **L.E. Rylaarsdam:** None. **A.D. Guemez-Gamboa:** None.

Poster

199. Genetic Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 199.19/A83

Topic: A.07. Developmental Disorders

Support: National Key R&D Program of China (No. 2017YFC1307500)
Shenzhen Overseas Innovation Team Project (No. KQTD20140630180249366)
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National Key R&D Program of China (No. 2018YFA0107203)
External Cooperation Program of Chinese Academy of Sciences (172644KYSB20160026)

Title: Autistic-like behaviors and atypical connectivity in SHANK3 mutant macaques

Authors: Y. ZHOU[1,8], J. SHARMA[2,3,4,9], Q. KE[10,11], *R. LANDMAN[12,1], S. ANTERAPER[5,3], M. SUR[3,6,4], H. ZHOU[8], A. P. XIANG[13,11], R. DESIMONE[14,3], G. FENG[7,1,12], S. YANG[15]; [1]McGovern Inst. for Brain Res., [2]Picower Inst. For Learning & Memory, [3]Dept. of Brain and Cognitive Sci., [4]Simons Ctr. for the Social Brain, [6]Picower Inst. for Learning and Memory, [7]Brain and Cognitive Sci., [5]MIT, Cambridge, MA; [8]Brain Cognition and Brain Dis. Inst., Shenzhen Inst. of Advanced Technol., Shenzhen, China; [9]9.Athinoula A. Martinos Ctr. for Biomed. Imaging, Dept. of Radiology,, Massachusetts Gen. Hosp., Charlestown, MA; [10]Ctr. for Stem Cell Biol. and Tissue Engin., Sun Yat-Sen Univ., Guangzhou, China; [11]Guangzhou Regenerative Med. and Hlth. Guangdong Lab., Guangzhou, China; [12]Stanley Ctr. for Psychiatric Res., Broad Inst., Cambridge, MA; [13]Ctr. for Stem Cell Biol. and Tissue Engin., Key Lab. for Stem Cells and Tissue Engin. (Sun Yat-Sen University), Guangzhou, China; [14]MIT, McGovern Inst. Brain Res., Cambridge, MA; [15]Col. of Vet. Med., South China Agr. Univ., Guangzhou, China

Abstract: Mutation or disruption of the *SHANK3 (*SH3 domain and ankyrin repeat) gene represents a highly penetrant, monogenic risk-factor for Autism Spectrum Disorder (ASD) and is a cause of Phelan–McDermid syndrome (PMS). Recent advances in gene editing have enabled the creation of genetically engineered non-human primate (NHPs) models, which might better approximate the behavioral and neural abnormalities of ASD than rodent models and lead to more effective treatments. Here, we report CRISPR/Cas9-mediated generation of germline-transmissible cynomolgus macaques and their F1 offspring carrying *SHANK3* mutations. Genotyping of somatic cells and brain biopsies confirmed mutations in the *SHANK3* gene and reduced SHANK3 proteins. Analysis of fMRI data revealed altered local and global connectivity patterns indicative of circuit abnormalities. The founder mutants exhibited sleep disturbances, motor deficits, and increased repetitive behaviors, as well as social and learning impairments. Together, these results parallel some aspects of the gene-circuit-behavior dysfunction in human ASD and PMS.

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Poster

199. Genetic Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 199.20/A84

Topic: A.07. Developmental Disorders

Support: Simons Foundation Autism Research Initiative Grant 345034

Title: Stereotyped behavior in rewarding scenarios in a mouse model of 16p11.2 hemideletion

Authors: ***G. R. ROJAS**[1], A. HELLER[3], A. BASTIN[1], A. DUERR[3], M. RITCHIE[2], N. M. GRISSOM[1];
[1]Dept. of Psychology, [2]Dept. of Neurosci., Univ. of Minnesota, Minneapolis, MN; [3]Dept. of Neurosci., Univ. of St. Thomas, Saint Paul, MN

Abstract: Neurodevelopmental disorders, including autism spectrum disorders, display strong male biases in diagnosis and severity, but how sex differences interact with genetic contributors to autism and leads to differential cognition is unclear. Our lab and others have previously found male-specific vulnerabilities in motivated behaviors in a mouse modeling 16p11.2 hemideletion, a copy number variation linked to neurodevelopmental diagnoses including autism. We tested 16p11.2 del/+ male and female mice in three rewarding scenarios to define how this genotype produces male-specific deficits in motivated behavior. First, male and female 16p11.2 del/+ animals are being tested in two discounting tasks (delay discounting and probability discounting/risky decision making) to assess reward motivation, impulse control, and outcome prediction. In pretraining, we replicate our previous finding of significantly reduced nonreinforced responding in del/+ males relative to wildtype males, even when reinforced responding is similar. This responding is equivalent between wildtype and del/+ females, and indicates that the amount of exploratory behavior del/+ males engage in is reduced, potentially contributing to delays in acquiring new motivated behaviors. Furthermore, these differences in exploratory behavior are most stereotyped when responses are cued, indicating del/+ male mice might be more sensitive to changes in the reliability of cues to signal the availability of reward. We are now testing the discounting tasks to determine if del/+ males reduce responding when predictability of reward delivery is uncertain (e.g. probability discounting), but not when the reward is certain and delayed (e.g. delay discounting). To establish how these cognitive behaviors are linked with neurobiology, we are probing catecholamine function in 16p11.2 del/+ mice with locomotor sensitization. At doses that induce significant locomotion in wildtype males, del/+ males are significantly less active and engage primarily in repetitive rotation. This

suggests that there are significant reductions and/or imbalances in dopamine function in the striatum which could drive both stereotypies/repetitive behaviors and the use of alternative neural circuits in reward-guided motivated behaviors.

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Poster

199. Genetic Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 199.21/A85

Topic: A.07. Developmental Disorders

Support: NIH Grant F049150

Title: ASXL3: Linking chromatin to convergent autism spectrum disorder biology

Authors: *B. MCGRATH[1], S. L. BIELAS[2], A. SRIVASTAVA[3], S. SALVI[1], R. KC[4]; [2]Dept. of Human Genet., [1]Univ. of Michigan, Ann Arbor, MI; [3]Sanjay Gandhi Postgraduate Inst. of Med. Sci., Lucknow, India; [4]Case Western, Cleveland, OH

Abstract: Autism spectrum disorder (ASD) is a complex brain disorder affecting 1 out of every 68 children in the United States. A pathological disruption of growth and organization of the cerebral cortex leads to ASD. Human genetic studies aimed at understanding the genetic etiology of ASD implicate chromatin as a nexus of convergent neuropathology. *De novo* truncating variants in *ASXL3* have been identified as a genetic basis of syndromic ASD. ASXL3 regulates the repressive chromatin modification H2A monoubiquitination (H2AUb1) which contributes to transcriptional plasticity pivotal to differentiation and acquisition of new cellular identities. We identified dysregulation of H2AUb1 as a key molecular pathology in primary cells derived from individuals with *ASXL3* variants. We generated *Asxl3* mice, expressing clinically relevant pathogenic variants to investigate ASXL3-dependent neuropathology. Initial findings showed a >50% reduction of layer 5 (L5) excitatory neurons, reduced corticospinal tracts, and increased levels of H2AUb1 in *Asxl3* null mice. These findings resemble the anatomy and cytoarchitecture described in ASD human post-mortem brains and implicate *Asxl3*-dependent mechanisms in the establishment of mature cortical neurons. However, the role of *Asxl3*-dependent H2AUb1 deubiquitination in regulating transcriptional profiles critical to fate decisions during cerebral cortex development have yet to be determined. We hypothesis that ASXL3-dependent deubiquitination activity is required for specifying NPC transcriptional programs critical for governing the neuronal diversity of the cortex. We pair single cell transcriptomics and epigenetic analysis of Neural progenitor cells (NPCs) to determine the *Asxl3*-dependent role in neuronal fate specification**.** Through unbiased single-cell RNA sequencing (scRNA-SEQ), we identify a

defect in excitatory neuron differentiation that can be attributed to transcriptional dysregulation of other ASD risk genes during corticogenesis. Taken together our findings implicate dynamic exchange of histone H2AUb1 as critical during mammalian brain development and key molecular pathology of ASD.

Disclosures: B. McGrath: None. **S.L. Bielas:** None. **A. Srivastava:** None. **S. Salvi:** None. **R. Kc:** None.

Poster

199. Genetic Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 199.22/A86

Topic: A.07. Developmental Disorders

Support: NRT Grant IGE1747486

Title: Genetic effects on domain-specific pathways that alter communication

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Abstract: Significant language and communication impairments have been observed in various neurodevelopmental disorders (NDD), including autism spectrum disorders (ASD) and Angelman syndrome (AS). Although high clinical and economic impacts incentivize identification of communication-specific-risk genes, this topic remains understudied. This is due to research barriers that include the complexity of language and communication, specifically in regard to whether difficulties arise from atypical social interactions, difficulties processing complex auditory information, cognitive impairments, motor deficits, or some combination. Significant overlap in the genetic architecture of NDDs further increases the difficulty in disentangling genetic contribution to communication/language-related phenotypes. Finally, limited research has addressed genetic effects on communication in non-human models. These barriers have limited our understanding of how genetics affect the neural systems associated with language and communication. Here we explore gene-brain-behavior relationships by studying how genes associated with NDDs alter expressive communication in two transgenic mouse models with mutations in rodent homologs for *UBE3A* and *CNTNAP2* (implicated in AS and ASD, respectively). Both mouse models show a reduction in ultrasonic vocalizations (USVs), mirroring human clinical findings. However, it is unlikely that this communicative reduction has a “common cause”. To test the hypothesis that different genes may influence different aspects of communication pathways, we examined the functional contribution to expressive communication skills in wildtype (WT) and transgenic mice (*Ube3a*, AS; *Cntnap2*, ASD). Results indicate that both motor skill and social activity significantly and positively influence net USV production in

WT mice (i.e., increased motor skills and increased social behavior result in more complex vocalizations). For our transgenic mice, we discovered different patterns of contribution for expressive USV production. In the mouse model of AS, only motor skill negatively impacted the production of complex vocalizations. In a mouse model of ASD, however, only atypical social behavior restricted complex vocalization production. Overall, our evidence shows that genes associated with language/communication-related NDDs may affect different domain-specific pathways that alter expressive communication. This novel approach to the study of communication in transgenic mice may improve early screening, and lead to gene-specific therapeutic interventions to improve communicative outcomes.

Disclosures: P.A. Perrino: None. **A.R. Rendall:** None. **R. Fitch:** None.

Poster

199. Genetic Models for Autism Spectrum Disorders

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Program #/Poster #: 199.23/B1

Topic: A.07. Developmental Disorders

Support: SFARI 564256
SFARI 513133

Title: Crispr activation rescues physiological deficits associated with scn2a haploinsufficiency

Authors: *P. W. SPRATT[1], S. TAMURA[2], C. KEESHEN[1], N. MATHARU[2], N. AHITUV[2], K. J. BENDER[1];
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Abstract: *De novo* mutations in the gene *SCN2A* are strongly associated with autism spectrum disorder (ASD) and intellectual disability. The majority of ASD-associated *SCN2A* mutations are protein truncating variants, resulting in conditions where individuals have only one functional copy of *SCN2A* (haploinsufficiency). *SCN2A* encodes the protein $Na_V1.2$, a voltage-gated sodium channel that is expressed throughout the brain, including neocortical excitatory neurons. Using a mouse model heterozygous for *Scn2a*, we found that $Na_V1.2$ loss resulted in developmentally distinct deficits in prefrontal cortex excitatory neurons. *Scn2a* haploinsufficiency impaired action potential initiation early in development, while a deficit in dendritic excitability persisted throughout life. These excitability deficits were associated with impaired excitatory synapses, even when *Scn2a* was disrupted late in development. These findings suggest that $Na_V1.2$ function is critical throughout life, raising the possibility that restoring normal $Na_V1.2$ function, even later in development, may result in a therapeutic benefit for individuals with ASD-associated *SCN2A* mutations.

To explore this possibility, we have developed CRISPR activation (CRISPRa) tools to increase the expression of the remaining functional *SCN2A* allele to normal physiological levels. CRISPRa targets a transcriptional activator (such as VP64), using a catalytically inactive Cas9 (dCas9), to regulatory elements of individual genes, upregulating their transcription. Using this approach, we optimized, in mouse Neuroblastoma-2a cells, a recombinant adeno-associated virus (rAAV) based system that upregulates *Scn2a* by targeting its promoter. We then injected this viral system into the prefrontal cortex at postnatal day 30. Four weeks post-injection, we found that *Scn2a* expression was increased and that features of intrinsic excitability and synaptic transmission were restored to normal levels. These results suggest that the restoration of *Scn2a* expression later in development can rescue cellular deficits associated with *Scn2a* haploinsufficiency, and further suggest that CRISPRa could be utilized as a potential therapeutic for other haploinsufficient genes in ASD and additional neurodevelopmental conditions.

Disclosures: P.W. Spratt: None. **S. Tamura:** None. **C. Keeshen:** None. **N. Matharu:** None. **N. Ahituv:** None. **K.J. Bender:** None.

Poster

199. Genetic Models for Autism Spectrum Disorders

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Title: Dynamic FoxG1 expression levels regulate autism associated behavioral circuits

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Abstract: Both up-regulation (duplication) and down-regulation (haploinsufficiency) of the transcription factor FoxG1 have been identified in syndromic forms of autism spectrum disorders (ASD). Furthermore, studies on idiopathic ASD patients have implicated FoxG1 dysregulation

more broadly in ASD etiology. Here we demonstrate that dynamic changes in FoxG1 levels at key transition points during differentiation are critical for proper circuit formation. In maturing circuits, either increased or decreased FoxG1 levels result in animals developing ASD-like social behavior impairments when both excitatory and inhibitory populations are affected. FoxG1 perturbation disrupts the excitation/inhibition balance during early juvenile development, prior to the emergence of ASD-related social and cortical EEG phenotypes. Indeed, FoxG1 augmentation selectively during the early juvenile period is sufficient to precipitate ASD-related phenotypes in wild type animals and markedly exacerbates the social impairments of FoxG1 haploinsufficient animals. Our findings illuminate the critical dosage and timing dependent requirements for FoxG1 in brain development and social behavior, and pinpoint the early postnatal period as being particularly sensitive to perturbations in FoxG1 levels.

Disclosures: G. Miyoshi: None. **Y. Ueta:** None. **A. Natsubori:** None. **H. Osaki:** None. **Y. Yagasaki:** None. **G.J. Fishell:** None. **R. Machold:** None. **M. Miyata:** None. **Y. Kishi:** None.

Poster

199. Genetic Models for Autism Spectrum Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 199.25/B3

Topic: A.07. Developmental Disorders

Support: NIH Grant 5R01MH108519-04
Gift Funds from Ms. Nancy Lurie Marks

Title: The effect of Dyrk1a mutations on neuronal growth, connectivity, and autism-relevant behaviors

Authors: *J. LEVY[1], D. T. PAGE[2];
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Abstract: Mutations in *DYRK1A* , which encodes a dual-specificity tyrosine kinase important for proliferation and differentiation in the developing brain, are associated with microcephaly in a subset of individuals with Autism Spectrum Disorder (ASD) and Intellectual Disability (ID). While the association of *DYRK1a* with microcephaly has been replicated in the literature, the cellular and molecular mechanisms underlying this phenotype are unknown. We have tested two competing hypotheses to understand how *Dyrk1a* mutations cause microcephaly: 1) decreased cell number, or 2) decreased cell size. To discriminate between these hypotheses, we have generated cortex-specific conditional heterozygous (cHet) and homozygous (cKO) mutants (*Emx1-cre*$^{+}$*;Dyrk1a*$^{loxP/+}$ and *Emx1-cre*$^{+}$*;Dyrk1a*$^{loxP/loxP}$, respectively) and applied the isotropic fractionator technique to obtain unbiased counts of neuronal and glial cell number and density in the developing and mature cerebral cortex, along with immunohistochemistry for cell-type

markers. These mutants display decreased brain and cortex mass throughout development and adulthood. Along with decreased cortex mass, we have found that both cortical neuron number and size are altered in an age and cell-type selective manner, with deep layer pyramidal neurons being particularly impacted during early postnatal development. Ongoing work is testing dysregulated protein synthesis as a potential mechanism for this effect and examining the relationship between cortical neuronal undergrowth and observed ASD-relevant behavioral phenotypes in *Dyrk1a* mutants.

Disclosures: **J. Levy:** None. **D.T. Page:** None.

Poster

199. Genetic Models for Autism Spectrum Disorders

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Topic: A.07. Developmental Disorders

Support: NIMH
Swebelius Foundation
Simons Foundation
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Title: An atlas-based approach to analysis of neural activity in scn1lab mutant zebrafish

Authors: *D. S. JIN[1], B. ROONEY[2], C. SAKAI[1], D. CASETTI[3], X. PAPADEMETRIS[3], E. J. HOFFMAN[1];
[2]Child Study Ctr., [3]Dept. of Biomed. Imaging, [1]Yale Univ., New Haven, CT

Abstract: Objective: Dravet syndrome is a pediatric condition characterized by severe intellectual disability and drug-resistant epilepsy. The *SCN1A* gene, which codes for the alpha subunit of the voltage-gated sodium channel NaV1.1, is disrupted in 70-80% cases of Dravet syndrome. In zebrafish, disruption of an ortholog, *scn1lab*, results in spontaneous seizures and nighttime hyperactivity. However, the brain activity correlates of *scn1lab* behavior have not been quantified at an anatomical level. Neural activity can be quantified using a normalized calculation of phosphorylated-extracellular signal-regulated kinase (pERK) and at specific regions using the Z-Brain atlas of the zebrafish brain. Here, we use a similar calculation with the Z-Brain atlas to highlight differences in baseline neural activity between *scn1lab* mutants and their wild-type counterparts.
Methods: We performed immunostaining for both pERK and total extracellular signal-related kinase (tERK) in zebrafish mutants carrying a 44 base pair deletion in the *scn1lab* gene (n=8)

and sibling-matched wild-type fish (n=5). Confocal images for both pERK and tERK were taken of each fish, and the relative fluorescence level of each image established at a voxel-by-voxel level. The pERK and corresponding tERK images were registered to a reference brain along with the Z-Brain atlas via nonlinear registration. The pERK images were then normalized according to the tERK images by dividing pERK image intensity by the corresponding tERK to create a standardized fluorescence value ratio (SFVR) to derive region-of-interest averages of brain activity.Results: We analyzed 210 individual regions of interest across our five aggregates. We found significant differences in volume-weighted SFVRs in three of aggregates (totaling 102 individual ROIs), and while two aggregates (totaling 108 individual regions of interest) showed no difference. This indicates that our method was able to quantify both differences and similarities in qualitative inspection of images. Across the 102 individual ROIs in the aggregates with significant differences, we are currently investigating neurotransmitters which may be altered, which will provide an opportunity to study mechanisms contributing to behavioral dysfunction in *scn1lab* mutants.
Conclusions: The normalization of pERK immunostaining provided a specific, quantified difference between brain activity in *scn1lab* mutant fish and their wild-type counterparts. The region-of-interest results of zebrafish *scn1lab* mutations could potentially be used to inform human electrophysiological and neuroimaging studies of individuals with Dravet syndrome.

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Poster

200. Small-Molecule Neurotransmitter Transport and Signaling

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 200.01/B5

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Title: Recovery of soman inhibited rat diaphragm by distinct bispyridinium compounds correlates with resensitizing effects in the neuronal human α7 nAChR

Authors: *T. SEEGER[1], C. SCHEFFEL[1], S. RAPPENGLÜCK[1], K. T. WANNER[2], F. WOREK[1], H. THIERMANN[1], K. V. NIESSEN[1];
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Abstract: Poisoning with organophosphorus compounds (OP) can lead to disturbance of numerous body functions and finally death due to respiratory failure. This is caused by the inhibition of acetylcholinesterase (AChE), leading to a pathophysiological increase of the neurotransmitter acetylcholine in cholinergic synapses. Basic treatment of OP poisoning with atropine is effective to counteract the toxic effects symptomatically at muscarinic receptors but

has no therapeutic effect at nicotinic receptors. Antagonizing the OP effects in target organs that are mediated by nicotinic receptors is only possible by the supplement of oximes able to reactivate OP-inhibited AChE. However, treatment with clinically used oximes is not sufficient in the case of poisoning by different nerve agents. Consequently, drugs directly interacting with nicotinic acetylcholine receptors (nAChR) are topics of interest. The 4-*tert*-butyl-substituted bispyridinium compound (BP) MB327 showed first promising therapeutic effects in *in vivo* (guinea pigs) (1). Further BP compounds, *tert*-butyl BP regioisomers MB327, PTM0001, PTM0002 and the methoxy BP regioisomers PTM0008, PTM0009 and PTM0010, were synthesized and tested on human α7 nAChR (hα7-nAChR) by automated whole-cell patch-clamp technique. Application of the agonist nicotine at a concentration of 100 µM, resulted in a hα7-nAChR induced cation influx. At higher nicotine concentrations (1 mM), the receptor current was inhibited due receptor desensitization (2). The above-mentioned BP compounds were able to abolish this desensitization ("resensitizing"). The mechanism was typical for a positive allosteric modulation type II (PAM type II). Rat diaphragm preparations were stimulated by indirect electric field stimulation technique (20, 50, 100 Hz). With increasing stimulation frequency the release of acetylcholine in the neuromuscular junction increased. Application of 3 µM soman led to a complete block of the muscle function. Application of MB327, PTM0001, PTM0002, PTM0008, PTM0009 and PTM0010 (1-300 µM) resulted in a partial restoration of soman inhibited muscle force. Hereby, the recovery of the muscle force was most pronounced at 20 Hz and 300 µM BP.
Further studies are needed to determine the mechanism of action in nicotinic subtyps as well as structure-activity relationships in more detail.
[1] C.M. Timperley et al., 2012. Med. Chem. Commun. 3, 352
[2] Scheffel, C., et al. 2018. Toxicol. Lett. 293, 149

Disclosures: T. Seeger: None. **C. Scheffel:** None. **S. Rappenglück:** None. **K.T. Wanner:** None. **F. Worek:** None. **H. Thiermann:** None. **K.V. Niessen:** None.

Poster

200. Small-Molecule Neurotransmitter Transport and Signaling

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 200.02/B6

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Title: *In vitro* pharmacological profiles of the bispyridinium non-oxime compound MB327 and its 2- and 3-regioisomers targeting cholinergic systems

Authors: *K. V. NIESSEN, S. RAPPENGLUECK, S. SICHLER, H. THIERMANN, F. WOREK, T. SEEGER;
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Abstract: Impairment of cholinergic neurotransmission is one of the most severe consequences of organophosphorus (OP) poisoning. OP compounds inhibit the enzyme acetylcholinesterase (AChE), resulting in accumulation of acetylcholine in the synaptic cleft. Following overstimulation of nicotinic (nAChRs) and muscarinic acetylcholine receptors (mAChRs) force receptor dysfunction and fatal disturbance of central und peripheral neuronal signaling up to respiratory arrest is the consequence. Current standard treatment, consisting of administration of a competitive mAChR antagonist (e.g. atropine) and an oxime (e.g. obidoxime, pralidoxime) for reactivation of inhibited AChE, is not sufficient in case of soman or tabun intoxications. An innovative approach comprises the use of compounds selectively targeting nAChRs, especially positive allosteric modulators, which increase the population of the conducting receptor state. MB327 (1,1'-(propane-1,3-diyl)bis(4-*tert*-butylpyridinium) di(iodide) is able to restore soman-blocked muscle-force partially in preparations of various species including human and was recently identified as "resensitizer". In further steps, the pharmacological profiles of MB327 and its 3-regioisomer (PTM0001) and 2-regioisomer (PTM0002) were comprehensively characterized in order to detect the effects on both respiratory muscle tissue and cholinergic receptors. Therefore, binding assays with nAChRs and mAChRs, functional studies by SSM (solid supported membranes) based electrophysiology, and *in vitro* muscle-force investigations of soman-poisoned rat hemidiaphragm preparations were performed. The results obtained from targets of different complexity (receptor, muscle tissue) showed that the pharmacological profiles of the 2- and 3-regioisomers were relatively similar to those of MB327. High concentrations (> 300 µM) showed inhibitory effects on muscle force, which might critically influence the application as an antidote. Furthermore, the affinity of MB327 at human mAChRs M_2 was in the micromolar range (pK_i 6.3 ± 0.5), which is described for the agonist acetylcholine (pK_i 6.5). On the other hand, PTM0001 and PTM0002 showed weak affinities (pK_i 4.6 ± 0.2 and 4.7 ± 0.2, respectively). Thus, more effective drugs have to be developed. Nevertheless, the combination of the methods presented is an effective tool to get insight into interactions on a molecular basis and to translate these effects to complex organ systems. T. Seeger et al., *Toxicology* **2012**, *294*, 80-84; K.V. Niessen et al., *Toxicol. Lett.* **2018**, *293*, 190-197

Disclosures: K.V. Niessen: None. **S. Rappenglueck:** None. **S. Sichler:** None. **H. Thiermann:** None. **F. Worek:** None. **T. Seeger:** None.

Poster

200. Small-Molecule Neurotransmitter Transport and Signaling

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 200.03/B7

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: CHOP Foerderer Grant for Excellence (HL)
R21NS072842 (DL and HL)

R01NS45986 (DL)
U54 HD086984 (DL)

Title: D-serine rescues cortical parvalbumin GABAergic deficits in alpha7 nicotinic acetylcholine receptor deletion models of schizophrenia

Authors: *H. LIN[1], F.-C. HSU[2], A. JACOBI[1], J. PANZER[1], D. COULTER[1], D. LYNCH[1]; [1]Children's Hosp. of Philadelphia and Univ. of Pennsylvania Perelman Sch. of Med., Philadelphia, PA; [2]Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: D-serine is an endogenous coagonist at the glycine site of synaptic NMDA receptors (NMDARs) and synthesized by serine racemase (SR) through conversion of L-serine. NMDAR hypofunction due to specific depletion of D-serine has been implicated in the pathogenesis of schizophrenia. Our previous studies have demonstrated that deletion of the α7 nicotinic acetylcholine receptor (nAChR) in mice leads to deficits of cortical SR/D-serine, glutamatergic synapses and parvalbumin (PV) GABAergic interneurons resembling schizophrenia. Here we report that D-serine treatment rescues cortical glutamatergic synaptic and PV GABAergic deficits in α7 knockout (α7-KO) cortical culture models. α7 nAChRs were localized and closely associated on the dendritic membranes of cortical PV GABAergic interneurons. Deletion of the α7 nAChR led to reduction of SR and D-serine in PSD-95-positive glutamatergic postsynaptic terminals on the dendrites of cortical GABAergic interneurons, suggesting SR/D-serine deficiency in glutamatergic synapses of α7-KO GABAergic interneurons. D-serine treatment rescued PV and glutamic acid decarboxylase 65/67 (GAD65/67) levels of PV GABAergic interneurons in α7-KO cortical cultures and organotypical prefrontal cortical slice cultures. In addition, NMDAR open-channel blocker MK-801 significantly reduced PV levels in cultured cortical PV GABAergic interneurons, resembling PV deficits in schizophrenia. Long-term effects of D-serine treatment largely reversed MK-801-induced PV reduction in α7-KO cultures, demonstrating that D-serine treatment can rescue cortical PV deficits *in vitro* and *in vivo*. Furthermore, D-serine treatment rescued glutamatergic synaptic deficits on cortical GABAergic interneurons in α7-KO cultures, suggesting that D-serine treatment may rescue cortical PV GABAergic deficits through rescuing glutamatergic synaptic deficits on PV GABAergic interneurons. Taken together, our findings demonstrate that D-serine treatment rescues cortical glutamatergic synaptic and PV GABAergic deficits in α7-KO models reminiscent of schizophrenia, thereby providing cellular and molecular mechanisms for D-serine treatment in schizophrenia.

Disclosures: **H. Lin:** None. **F. Hsu:** None. **A. Jacobi:** None. **J. Panzer:** None. **D. Coulter:** None. **D. Lynch:** None.

Poster

200. Small-Molecule Neurotransmitter Transport and Signaling

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 200.04/B8

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: CNPq
CAPES
FAPERJ

Title: Caffeine regulates GABA uptake via adenosine receptor blockage and cAMP signaling in the developing avian retina

Authors: *V. P. P. BORGES-MARTINS[1], D. D. P. FERREIRA[3], A. C. SOUTO[1], J. G. NETO[1], D. PEREIRA-FIGUEIREDO[2], K. C. CALAZA[2], K. J. OLIVEIRA[1], A. C. MANHAES[4], R. A. M. REIS[3], R. C. C. KUBRUSLY[1];
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Abstract: Introduction: Caffeine (caf) is the most consumed psychostimulant drug in the world, affecting several behavioral, cognitive and physiological functions in the CNS. Pharmacologically, caf acts as a non-selective antagonist of adenosine A_1 and A_{2A} receptors (A_1R and $A_{2A}R$), which are responsible for a plethora of actions during development and are widely expressed in retina layers. **Objective:** We aim to investigate whether long term caf exposure is able to modify GABA uptake in the avian retina and to evaluate the mechanisms involved in this process. **Methods:** White Leghorn chicken embryos aged 11 days (E11) were given a single injection (in ovo) of caf 100µM in the air chamber and left until E15. The animals were sacrificed and the retina dissected for neurochemicals assays of [^{3}H]-GABA uptake and Western Blotting. One-way or two-way ANOVA followed by Bonferroni post hoc was performed for results with 3 or more groups and for pairwise comparisons, Student's t-test was used. The results were expressed as mean ± SEM and statistical significance was reached when $p<0.05$. All experiments were approved by the Committee on Animal Research and Ethics of The Federal University of Rio de Janeiro (#IBCCF035). **Results:** We determined GABA transport equilibrium at both basal (C) and caf conditions by measuring [^{3}H]-GABA uptake at different time points (1, 3, 5, 10, 30, 60, 120 and 180 min) (C: V_{max}=302 K_m=18.8, caf: V_{max}=542.3 K_m=105.6). Our results showed that [^{3}H]-GABA transport was dependent on Na^+ and blocked at 4ºC or by NO-711 (C= 329.1±31.56, w/o Na^+= 26.67±2.85, 4ºC= 20±6.08, NO-711= 53.67±3.71). Additionally, GAT-1 ontogenesis was analyzed during E11-E15 (E11= 0.07±0.004, E12= 0.075±0.013, E13= 0.07±0.004, E14= 0.05±0.001, E15= 0.09±0.004). [^{3}H]-

GABA uptake was measured at E15 after 1, 24 or 96h of caf injection. Caf decreased GABA uptake only after 96h (C= 1±0.047, 1h= 1.15±0.035, 24h= 1.1±0.11, 96h= 0.55±0.07). GAT-1 protein levels increased after treatment with caf (C= 1±0.15, caf= 1.5±0.12), which also increased A_1R protein content (C= 1±0.17, caf= 2±0.29). The decrease in GABA uptake promoted by caf was reverted by CHA, CGS 21680 and H-89, which were administered for 15 min before the assay (C= 330±30, caf= 180±20, CHA= 320±30, caf+CHA= 300±40, CGS= 290±20, caf+CGS= 340±15, H-89= 440±5, caf+H-89= 450±85). **Conclusion:** GAT-1 is expressed evenly throughout the treatment period and is responsible for most of GABA uptake in the retina, which is Na^+- and temperature-dependent. Caf reduced GABA uptake, but increased GAT-1 protein levels after 96h. A_1R protein levels were also increased. The decrease in GABA uptake was reverted by a PKA inhibitor or A_1R and $A_{2A}R$ agonists.

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Poster

200. Small-Molecule Neurotransmitter Transport and Signaling

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Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: NSERC Grant: RGPIN-2019-04925

Title: Characterization of N2a neuroblastoma cells as a model of ATP and catecholamine co-release

Authors: *G. J. JENSEN, B. KIM, A. DOS SANTOS, D. T. POBURKO;
Biomed. Physiol. and Kinesiology, Simon Fraser Univ., Burnaby, BC, Canada

Abstract: Sympathetic nerves release neurotransmitters and neuropeptides to stimulate vascular smooth muscle cells contraction in order to regulate vascular tone. While the functional regulation of co-release is well characterized, the underlying molecular mechanisms are largely unclear. We recently reported that ATP and norepinephrine co-release relies on separate pools of vesicles in the sympathetic nerves surrounding the rat tail artery and that each vesicle type preferentially associates with specific calcium channels. However, these intact tissues have significant limitations for molecular analyses. Thus, we investigated the N2a cell line as a model of ATP and catecholamine co-release. RT-PCR and immunostaining show these cells to express the vesicular nucleotide transporter (VNUT) and vesicular monoamine transporter-2 (VMAT-2) that load ATP and catecholamines (eg. NE or dopamine) into synaptic vesicles. We generated genetically encoded reporters of the release of ATP and catecholamine containing vesicles

(VMAT2-pHiji and VNUT-pHluorin) to detect electrically evoked vesicle release. As in tail artery, immunocytochemistry shows anti-colocalization of VNUT and VMAT2 containing vesicles in axon-like N2a neurites. We then assessed the potential colocalization of VNUT with the lysosomal marker LAMP-1, based on others' finding in epithelial cells. A significant fraction of small, LAMP-1 containing puncta in cell bodies and neurites colocalize with VNUT but not with VMAT2. We also observed differential accumulation of VNUT, VMAT and LAMP-1 within neurites, varicosities and the soma of the N2a cells. Further, immunocytochemical analysis of MAP-2 and Tau, proteins typically used to characterize dendrites and axons, respectively, demonstrated the nature of the neurites in which we identified accumulations of VNUT and LAMP-1. These findings suggest that the N2a cell line is a promising model to further characterize molecular aspects of the ATP and catecholamine co-release that is essential to sympathetic regulation of the vasculature. This model will accelerate proof of principle discoveries to be further validated in intact tissue.

Disclosures: G.J. Jensen: None. **B. Kim:** None. **A. dos Santos:** None. **D.T. Poburko:** None.

Poster

200. Small-Molecule Neurotransmitter Transport and Signaling

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 200.06/B10

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: Faperj
CNPq
Capes

Title: L-arginine modulates calcium currents and downstream signaling pathways via nitric oxide in developing avian retinal cells

Authors: *R. PAES-DE-CARVALHO, I. DOMITH, T. A. MEJÍA-GARCÍA, A. T. DUARTE-SILVA;
Neurobio., Fluminense Federal Univ., Niterói, Brazil

Abstract: Nitric oxide (NO) is an important non-classical neurotransmitter or neuromodulator in the CNS, being produced from L-arginine (L-arg) in a reaction catalyzed by NO synthases (NOS). In the retina, NO can activate different signaling pathways leading to ERK, AKT as well as CREB stimulation and promotes neuronal survival in purified cultures of avian retinal neurons. Ca^{++} ions control diverse metabolic signals through cell entry by channel opening or modulation of different transport mechanisms. Ca^{++} signals can be initiated by binding to several proteins such as calmodulin and activation of Ca^{++}/calmodulin-dependent enzymes such as CAM kinases (CAMK) or NOS. Here we report that L-arg activates Ca^{++} currents and AKT

phosphorylation in retinal cells. Chick embryo retinal cells in culture were transfected with pGP-CMV-GCaMP6f and Ca^{++} influx was analyzed in a confocal microscope after stimulation with L-arg. A striking increase of Ca^{++} influx was observed after L-arg application. This effect was concentration-dependent attaining a maximal in 1 mM and was inhibited by the NOS inhibitor 7-nitroindazole (100 µM) or the L-type Ca^{++} channel blocker nifedipine (100 µM). The increase of Ca^{++} influx was also observed when cells in culture were pre-loaded with $^{45}Ca^{++}$ and stimulated with L-arg or the NO donor SNAP, both effects being blocked by nifedipine. In western blot experiments using the same culture system we have previously shown that L-arg or SNAP stimulates AKT^{Ser473} and AKT^{Thr308} phosphorylation in a way dependent on the classical NO/cGMP/cGK and the PI3K pathways. Interestingly, this effect was abrogated by Ca^{++} chelators EGTA or BAPTA-AM, calmodulin inhibitors calmidazolium, W7 or trifluoperazine or the CAMK inhibitors KN62 or KN93. The AKT stimulation by YC1, a direct guanylyl cyclase stimulator, was also blocked by EGTA or KN62, suggesting that the Ca^{++} effect is in a step downstream to activation of the NO/cGMP/cGK pathway. Accordingly, we found an increase of Phospho-$CaMKII^{Thr286}$ immunofluorescence when L-arg or SNAP were added to cultures. We suggest a model in which L-arg increases NO production and cGK stimulation which then phosphorylates L-type Ca^{++} channels allowing Ca^{++} influx, activation of CaMKII and stimulation of downstream pathways including PI3K/AKT.

Disclosures: R. Paes-de-Carvalho: None. **I. Domith:** None. **T.A. Mejía-García:** None. **A.T. Duarte-Silva:** None.

Poster

200. Small-Molecule Neurotransmitter Transport and Signaling

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Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: The Beijing Municipal Science & Technology Commission (Z181100001318002)
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Title: Genomewide screen for synaptic vesicle transporters - Identification of SLC35D3 as a vesicular UDP - glucose transporter

Authors: C. QIAN[1,2], Z. WU[1,2], R. SUN[1,2], H. YU[3,2], J. ZENG[3,2], ***Y. LI**[1,2,3,4];
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Acad. for Advanced Interdisciplinary Studies, Beijing, China; [4]Chinese Inst. for Brain Res., Beijing, China

Abstract: Vesicular neurotransmitter transporters (VNTs) mediate selective uptake and enrichment of small molecule neurotransmitters into synaptic vesicles (SVs), and are therefore one major determinant of synaptic output for specific neurons. All the known VNTs belong to solute carrier (SLC) transporter family. Given that SLC contains ~ 450 members, with more than half of which are still orphan or poorly characterized, we hypothesize there exist novel VNTs in the SLC family, and their cognate substrates are potential new neurotransmitters or neuromodulators. To identify putative new SV transporters, we carried out genome-wide localization screen in cultured neurons. We systematically cloned 360 (out of 452 in total) human SLC genes to fuse them in frame with a fluorescent protein gene. We then co-expressed SLC with a known SV marker as well as other organelle markers. Using known VNTs' localization score as a benchmark, we identified ~30 novel transporters capable of localizing to SVs. To validate their *in vivo* localization, we immuno-isolated native SVs from mouse brains. Proteomic analysis revealed the presence of 7 (out of 30) transporters, with a subset of orphan SLC35 subfamily transporters, SLC35D3, SLC35F1 and SLC35G2. Further ultrastructural analysis by electron microscopy confirmed SLC35D3's ability to localize on SVs. Using mass spectrometry-based metabolite profiling and radioactive transport assay, we identified and confirmed UDP-glucose, a nucleotide-sugar, as a specific substrate for SLC35D3. Finally, we performed radioactive transport assay using native SVs derived from mouse brain, and observed UDP-glucose, but not its close analog UDP-galactose, was able to be selectively uptaken into SVs in a way sensitive to vesicular proton electrochemical gradient. In sum, our genome-wide analysis identified unexpected rich vesicular transporter candidates and among them SLC35D3 as a novel vesicular UDP-glucose transporter. These efforts would likely yield new insights to the function of SLC family genes and provide better understanding of the molecular diversity of chemical neurotransmitters.

Disclosures: C. Qian: None. **Z. Wu:** None. **R. Sun:** None. **H. Yu:** None. **J. Zeng:** None. **Y. Li:** None.

Poster

200. Small-Molecule Neurotransmitter Transport and Signaling

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Program #/Poster #: 200.08/B12

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: NINDS F99NS105208
NIMH R21MH108867
NIMH R21117434

Title: Abnormal copper transporter CTR1 in postmortem schizophrenia hippocampus: A subregion and laminar analysis

Authors: R. C. ROBERTS[1], C. B. FARMER[2], C. MORGAN[2], V. SINHA[2], L. ODOM[2], *K. E. SCHOONOVER[3];
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Abstract: The dystrobrevin binding protein 1 (*DTNBP1*) gene encodes the dysbindin-1 protein family and is a top candidate gene for schizophrenia. Dysbindin-1 modulates copper transport that is crucial for myelination, monoamine metabolism, and cellular homeostasis. Schizophrenia patients (SZP) exhibit increased plasma copper, lower levels of copper and decreased copper enzyme (CTR1), responsible for copper transport across the blood brain barrier, in the substantia nigra.
Several schizophrenia brain regions exhibit decreased dysbindin-1 including the hippocampus. We hypothesized that there would be less dysbindin-1 and CTR1 levels in this brain region in SZP and performed a quantitative study using immunohistochemistry on controls and SZP (n=10 per group).
The hippocampus proper, dentate gyrus, entorhinal cortex, and subiculum were richly immunolabeled for CTR1 in both controls and SZP. Qualitatively, CTR1 immunolabeling was present in the same cell types in both groups. Only CTR1 immunolabeling in the entorhinal cortex and dentate gyrus showed significant differences between groups. In the superficial area of the entorhinal cortex, both neuropil and cells showed lower optical density values in the SZP than in controls. The opposite pattern was observed in the molecular layer of the dentate gyrus, where SZP had significantly higher optical density values than did controls. The density and distribution of dysbindin-1 immunolabeling was similar between groups with no significant differences. These results suggest laminar specific alterations of copper transport within SZP that is not directly caused by decreased dysbindin-1. Therefore, dysbindin-1 may be sufficient but not necessary to induce abnormal copper transport in SZP.

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Poster

200. Small-Molecule Neurotransmitter Transport and Signaling

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 200.09/B13

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: NINDS R01-NS092716
NSF DGE-1633213

NSF Graduate Research Fellowship

Title: An evolutionary approach towards decoding the role of endocannabinoids in pain modulation

Authors: *R. T. PAULSEN, E. J. KABEISEMAN, B. D. BURRELL;
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Abstract: Chronic pain has emerged as a prominent global health challenge, with an estimated 1.5 billion adults suffering from pain worldwide, and half of these cases presented as chronic. The development of therapeutics is problematic because chronic pain can involve the nociceptive (pain signaling) and non-nociceptive neural circuitry networks. A potential treatment route for chronic pain could be to exploit the interdependent neuromodulation of pain, learning and memory. However, this approach is limited by an incomplete understanding of their overlapping cellular mechanisms. Endocannabinoids (eCBs) are lipid neurotransmitters which have been found to modulate the non-associative learning processes that accompany pain. However, the physiological role that eCBs play on the pro- to anti-nociceptive continuum has been largely unexplored and must be investigated before effective cannabinoid therapies can be implemented. Additionally, opposing effects of cannabinoid therapies have been documented in pre-clinical and clinical trials, indicating that the effects of cannabinoids in pain signaling require more investigation at the basic mechanism level. We are using the medicinal leech, *Hirudo verbana*, to study eCB-induced synaptic plasticity and its connection to nociception-related learning because of the intrinsic accessibility and conserved arrangement of its neurons for electrophysiological and molecular manipulation. Here, we present the first draft genome of *Hirudo* and discuss sequencing efforts probing for genes to validate the draft genome, *Hirudo* eCB receptor targets, and associated enzymes. Quantitative reverse transcription PCR experiments with diacylglycerol lipase (accession #KU500007) to investigate the activity-dependent modulation of eCB synthesis will be discussed. Furthermore, we have found sequences that appear to be conserved orthologues of fatty acid amide hydrolase 1 (FAAH1) and transient receptor potential vanilloid (TRPV) receptors, as well as other channels in the TRP family. In addition, we have found evidence of metabotropic receptors in *Hirudo* that may either be related to cannabinoid receptors (CB1 or CB2), or to orphan G-Protein Coupled Receptors (GPCRs) recently reported to respond to cannabinoids.

Disclosures: R.T. Paulsen: None. **E.J. Kabeiseman:** None. **B.D. Burrell:** None.

Poster

200. Small-Molecule Neurotransmitter Transport and Signaling

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 200.10/B14

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: Southern Illinois University Edwardsville

Title: Correlating internal alcohol concentration with splicing and behavior in *drosophila*

Authors: *E. HAUSMAN[1], E. PETRUCCELLI[2];
[2]Neurosci., [1]Southern Illinois Univ. Edwardsville, Edwardsville, IL

Abstract: Alcohol abuse is a persistent determent to society, directly affecting an individual's behavior and metabolism, and contributing to nearly 4% of deaths worldwide. However, the mechanisms by which different levels of alcohol changes gene expression in specific neurons are not well understood. *Drosophila melanogaster* has been a good model for better understanding how alcohol affects metabolism and alcohol-related behavior. Recent work in our lab has shown that alcohol can alter transcript splicing in the fly's memory-encoding neurons. Here, we establish a protocol for measuring the internal ethanol concentration of wildtype flies exposed to different treatments of vaporized ethanol. We plan to correlate these internal concentrations with specific splicing events and alcohol-induced behaviors. We also will use RNAi to knockdown candidate spliceosome genes, such as Cdc5, to examine whether impaired splicing influences alcohol-induced behaviors such as sedation resistance or ethanol absorption/metabolism. Our findings could lead to the study of other related splicing factors and help resolve the mechanism by which alcohol influences splicing. Due to the high conservation of spliceosomal complexes between flies and humans, these experiments will help us better understand how alcohol affects the transcriptional state of the human brain.

Disclosures: E. Hausman: None. **E. Petruccelli:** None.

Poster

200. Small-Molecule Neurotransmitter Transport and Signaling

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 200.11/B15

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: URCA, Southern Illinois University Edwardsville

Title: Function of *Drosophila* CG9098 in alcohol-induced sedation response

Authors: *K. LAHUE[1], E. PETRUCCELLI[2];
[2]Neurosci., [1]Southern Illinois Univ. Edwardsville, Edwardsville, IL

Abstract: The use of alcohol is known to cause many different effects on behavior. The effects of alcohol on behavior can easily be studied in *Drosophila melanogaster*, which is an ideal genetic model species. Like humans, flies can form associative preference for sensory cues that were previously paired with intoxication experience. Recent RNA sequencing identified genes

that changed in expression when flies associated intoxication with sensory cues. One candidate gene, of unknown function, was *CG9098. CG9098* is located on the second chromosome, expressed at high levels during development, and is predicted to be involved in cellular signaling. However, its specific role in behavioral response to alcohol has not been investigated. We hypothesized that flies with reduced *CG9098* expression have an altered resistance to alcohol sedation when exposed to ethanol vapor. We examined $CG9098^{MB09740}$ mutant flies and flies expressing *CG9098-RNAi* only in neurons. Preliminary results suggest that $CG9098^{MB09740}$ mutants are more resistant to ethanol-induced resistance, whereas pan-neuronal *CG9098* knockdown has no effect on sedation sensitivity. The variation between these results suggests that further research should be done to determine the temporal and cell-type specific requirement for CG9098 in alcohol-associated behaviors. In the future, other candidate genes can similarly be tested to elucidate the function of CG9098 and further identify novel mechanisms underlying conserved alcohol-induced behavior.

Disclosures: K. Lahue: None. **E. Petruccelli:** None.

Poster

200. Small-Molecule Neurotransmitter Transport and Signaling

Location: Hall A

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Support: William Paterson University
NIA/NIH intramural Research Program
CONACYT#CVU332502/232728
NIH Grant DA032890

Title: Behavioral modifications and alcohol preference following deletion of type two cannabinoid receptors in dopamine neurons and microglia

Authors: *E. S. ONAIVI[1], A. CANSECO-ALBA[2], B. SANABRIA[1], T. ROHANI[1], M. ZAMORA[1], S. ANGARITA[1], S. GOMEZ[1], J. LOUIS[1], J. BEJARANO[1], S. SGRO[1], A. TAGLIAFERRO[1], S. M. BIERBOWER[1], K. MARTIN[1], H. ISHIGURO[3], Q.-R. LIU[4]; [1]Dept. of Biol., William Paterson Univ., Wayne, NJ; [2]Pharmacobiology, Cinvestav-IPN, Ciudad DE Mexico, Mexico; [3]Univ. of Yamanashi, Chuo, Yamanashi, Japan; [4]LCI/NIA/NIH, Baltimore, MD

Abstract: CB1 and CB2 cannabinoid receptors (CB1Rs, CB2Rs) are expressed in neurons and neuroglia cells, and CB2Rs are enhanced during inflammation. CB2Rs expressed in neurons and glial cells are emerging as components of neuroinflammation and key regulators of immune response. We used DAT-*Cnr2* and Cx3cr1-*Cnr2* conditional knockout (cKO) mice with deletion

of CB2Rs from dopamine (DA) neurons and microglia respectively, with C57BL/6Js as wild type (WT) controls. We utilized Immunoblotting, immunohistochemistry, behavioral assessment in models of traumatic brain injury (TBI), alcohol, preference, consumption and assessment of the relative abundance of *Akkermansia muciniphila* in the gut-microbiome to determine the microbiome-neuro-immuno-modulatory effects of CB2Rs. We report that CB2Rs are involved in the tetrad assay induced by cannabinoids in the WT and the CB2R cKO mice contrary to the long-standing notion that the characteristic tetrad tests were induced mainly by CB1R agonism. In the hippocampus, there was enhanced IBA1 immunoreactivity in both CB2R cKO mice, and CD11b detected microglia activation in the dentate gyrus in WT, DAT-*Cnr2* and Cx3cr1-*Cnr2* cKO mice with clear morphological difference in the Cx3cr1-*Cnr2* cKO mice after stress. We found that neuroinflammation signaling pathways of PI3K/AKT/mTOR, MAP/ERK and NF-κB were differentially affected by the cell-type specific deletion of CB2R in cerebral cortexes of CB2R cKO and WT mice. The quantification and analysis of Akkermansia *muciniphila* - a potential indicator species for an anti-inflammatory mouse-gut microbiome, supported microbiome-neuro-immuno-modulatory effects of CB2Rs. Mice with deletion of CB2Rs in microglia were more sensitive that those with deletion of CB2Rs from DA neurons compared to WT controls during induction of microglia activation using TBI, Poly IC or lipopolysaccharide (LPS). Alcohol preference ratio was significantly higher in Cx3cr1-*Cnr2* cKO and WT, than DAT-*Cnr2* cKO mice that consumed less alcohol. WT mice and Cx3cr1-*Cnr2*, but not DAT-*Cnr2* cKO mice showed robust conditioning to alcohol in the CPP paradigm. Surprisingly Cx3cr1-*Cnr2* cKO that had higher alcohol preference showed reduced alcohol preference after TBI. The results from our studies indicate that CB2R microbiome-neuro-immune signaling is associated with the behavioral and morphological modifications and alcohol preference following the deletion of CB2Rs from DA neurons and microglia. In conclusion, investigation of CB2R neuro-immune signaling in gut-brain-axis will contribute to understanding psychiatric and neurological disorders that are associated with neuroinflammation.

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Poster

200. Small-Molecule Neurotransmitter Transport and Signaling

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Program #/Poster #: 200.13/B17

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: University of Illinois at Urbana-Champaign
NIH R21NS085665

Title: Simultaneous electrical and chemical transmission recording at the nanoscale

Authors: *M. SHEN[1], R. CHEN[2], J. STARCEZ[2], S. PHAM[2];
[1]Chem., Univ. of Illinois At Urbana-Champaign, Urbana, IL; [2]Chem., Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: Nanoscale in-vivo studies on the signaling of a broad range of neurotransmitters are essential to understand brain functions and diseases. Shen group have developed versatile nanopipette electrodes to enable the detection and quantification of small molecule neurotransmitters, such as acetylcholine (*1-4*). Recently, we have reported studying chemical transmission with nanometer resolution at single synaptic cleft and single cell levels, respectively (*5, 6*). We employed scanning electrochemical microscopy (SECM) (*7, 8*) for accurate positioning of our nanoprobes with nm spatial resolution. Our results showed that our nanoprobes, with size as small as 15 nm in radius, can detect and quantify acetylcholine neurotransmission in real time, with nm spatial resolution and high signal to noise ratios. Our single synaptic study unveiled the diverse chemical transmission dynamics of cholinergic transmitters, composed of singlets, doublets and multiplets, at single synaptic cleft (*6*). Additionally, we have created an analytical method to measure vesicle density concurrently with chemical transmission in living cell conditions (*5).*
Here, I will present our latest efforts towards simultaneous electrical and chemical neurotransmission recording with nm spatial resolution. The nano-electroanalytical platform we have developed is enabling a variety of new measurements on signaling dynamics across a diverse range of length scales, i.e. at single cells and at single synapses, and will create exciting opportunities in studying transmission from various neuronal models and in our understanding of neurological disorders from a distinctive perspective.
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(1)Iwai, N. T.; Kramaric, M.; Crabbe, D.; Wei, Y.; Chen, R.; **Shen, M.*** Anal. Chem., **2018**, *90*, 3067-3072.
(2)Shen, M.*; Colombo, M. L. Analytical Methods, **2015**, *7*, 7095-7105.
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(5)Welle, T. M.; Alanis, K.; Colombo, M. L.; Sweedler, J. V.; **Shen, M.*** Chem. Sci., **2018**, *9*, 4937-4941.
(6)**Shen, M.;*** Qu, Z.; Deslaurier, J.; Welle, T.; Sweedler, J.; Chen, R. J. Am. Chem. Soc., **2018**, *140*, 7764-7768.
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(8) **Shen, M.**; Ishimatsu, R.; Kim, J.; Amemiya, S. J. Am. Chem. Soc., **2012**, *134*, 9856-9859.

Disclosures: **M. Shen:** None. **R. Chen:** None. **J. Starcez:** None. **S. Pham:** None.

Poster

200. Small-Molecule Neurotransmitter Transport and Signaling

Location: Hall A

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Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: HD 045022
R37-CA084198
NS088538
MH104610
MH085802-06
SCSB award# 2389069
RSRT grant 50-1873-0201

Title: Identification of KCC2 expression enhancer compounds as a basis for treatment of Rett syndrome

Authors: *X. TANG[1], J. DROTAR[1], K. LI[2], C. CLAIRMONT[1], S. BRUMM[3], A. SULLINS[2,4], H. WU[1], S. LIU[1], J. WANG[4], N. GRAY[4,3], M. SUR[2], R. JAENISCH[1,2];
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Abstract: The delicate balance between excitatory and inhibitory signaling in neural circuits (E/I balance) is critical for brain function. Disruption in E/I balance and hyperexcitability at the synapse, neural circuit, and behavioral levels have emerged as core mechanisms underlying a variety of brain disorders. The neuron-specific K+/Cl- cotransporter-2 (KCC2) is a 'keystone' molecule that is critical for the maturation of both GABAergic neurotransmission and excitatory synapse function, and has emerged as a promising therapeutic target to restore E/I balance in brain disorders including epilepsy, schizophrenia, spinal cord injury, and Rett syndrome, a severe neurodevelopmental disorder. Due to the lack of robust high-throughput screening (HTS) assay, it has been challenging to discover chemical compounds that enhance the expression of the KCC2 gene. In this study, we report the development of a novel human neuron-based high-throughput drug screening platform that allows for the rapid assessment of KCC2 gene expression in genome-edited reporter neurons. We have identified a group of compounds from an unbiased screen of over 900 small molecule chemicals that enhance KCC2 expression termed KCC2 expression-enhancer compounds (KEECs). The identified KEECs include FDA-approved drugs that are inhibitors of the FLT3 or GSK3β kinase pathways, and activators of the SIRT1 or TRPV1 pathways. We demonstrate that treatment with these hit compounds robustly increases KCC2 expression in human WT and isogenic Methyl CpG binding Protein 2 (*MECP2*) mutant

RTT neurons, and rescues the deficits in GABA reversal potential, excitatory synaptic transmission, and morphological development of RTT neurons to levels equivalent to WT neurons. Moreover, injection of KEECs KW-2449 or Piperine into a Mecp2 mutant mouse model of RTT ameliorates disease-associated respiratory and locomotion abnormalities. The small molecule compounds described in our study could potentially benefit various brain diseases through a novel mechanism of enhancing KCC2 expression.

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Poster

200. Small-Molecule Neurotransmitter Transport and Signaling

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NIH Grant AG062129
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Title: CT1812 demonstrates evidence of synapse preservation in Alzheimer's disease patients and abeta oligomer displacement in preclinical models

Authors: *S. M. CATALANO[1], K. MOZZONI[1], C. REHAK[1], L. WAYBRIGHT[1], K. SADLEK[1], H. SAFFERSTEIN[1], E. WATTO[1], N. IZZO[1], M. GRUNDMAN[2], S. DEKOSKY[3], L. SCHNEIDER[4];
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Abstract: CT1812 is a disease-modifying sigma-2 receptor antagonist currently in clinical trials in Alzheimer's patients (AD). Preclinical evidence indicates that sigma-2 receptors regulate the Abeta oligomer receptor complex on neurons; individual sigma-2 constituent proteins play a role in lipid metabolism, protein/lipid membrane trafficking, growth factor signaling and autophagy. CT1812 allosterically modulates the sigma-2 complex, which in turn destabilizes the oligomer binding site within the oligomer receptor complex, resulting in an increased off-rate of oligomers. CT1812 results in increases oligomer concentration within the interstitial brain fluid,

as well as in the cerebrospinal fluid (CSF), consistent with clearance from the brain. CT1812's displacement of oligomers from their receptor returns oligomer-induced toxic changes to normal. CT1812 restores membrane and protein trafficking deficits, stops spine and synapse loss *in vitro*, and improves cognitive deficits in transgenic mouse AD models. A phase 1a/2b clinical trial of CT1812 was conducted in mild to moderate AD patients where participants received one of three doses of CT1812 or placebo once daily for 28 days. Plasma and CSF protein, lipid and metabolite values were measured at baseline and at 28 days via ELISA or tandem mass spec, and the amount of change of each analyte within each patient over the course of the trial was calculated. Treatment group averages or medians were analyzed to determine statistical differences between the placebo and pooled drug-treated groups. CSF concentrations of Abeta oligomers were increased in CT1812-treated and decreased in placebo-treated patients; the difference between the groups was significant. Many plasma and CSF proteins, lipids and metabolites are dysregulated in AD patients vs. age-matched cognitively normal individuals. Biofluid concentrations of large numbers of these analytes were changed in a therapeutic direction in CT1812-treated but continued to worsen in placebo-treated patients; the difference between the groups was significant for 30 CSF proteins and 91 plasma proteins and lipids. CSF concentrations of synaptic protein fragments neurogranin and synaptotagmin were significantly decreased in CT1812-treated but continued to increase in placebo patients; the difference between the groups was significant. Together this clinical evidence is consistent with preclinical data, supports CT1812's mechanism of action, and provides encouraging evidence of target engagement, reduction of synaptic damage and disease-modification. Additional Phase 2 six-month trials in this patient population are currently underway.

Disclosures: S.M. Catalano: A. Employment/Salary (full or part-time):; Cognition Therapeutics, Inc. **K. Mozzoni:** A. Employment/Salary (full or part-time):; Cognition Therapeutics, Inc. **C. Rehak:** A. Employment/Salary (full or part-time):; Cognition Therapeutics, Inc. **L. Waybright:** A. Employment/Salary (full or part-time):; Cognition Therapeutics, Inc. **K. Sadlek:** A. Employment/Salary (full or part-time):; Cognition Therapeutics, Inc. **H. Safferstein:** A. Employment/Salary (full or part-time):; Cognition Therapeutics, Inc. **E. Watto:** A. Employment/Salary (full or part-time):; Cognition Therapeutics, Inc. **N. Izzo:** A. Employment/Salary (full or part-time):; Cognition Therapeutics, Inc. **M. Grundman:** F. Consulting Fees (e.g., advisory boards); Cognition Therapeutics, Inc. **S. Dekosky:** F. Consulting Fees (e.g., advisory boards); Cognition Therapeutics, Inc. **L. Schneider:** F. Consulting Fees (e.g., advisory boards); Cognition Therapeutics, Inc..

Poster

200. Small-Molecule Neurotransmitter Transport and Signaling

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 200.16/B20

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: NIH Grant AG062129

Title: Alzheimer's disease therapeutic target sigma-2 receptor directly interacts with PGRMC1 in primary cultured neurons

Authors: *K. T. SADLEK, N. J. IZZO, C. REHAK, R. YURKO, L. WAYBRIGHT, E. WATTO, N. KNEZOVICH, K. MOZZONI, H. SAFFERSTEIN, S. M. CATALANO; Cognition Therapeutics, Inc., Pittsburgh, PA

Abstract: CT1812 (Elayta^{TM}) is a disease-modifying sigma-2 receptor antagonist currently in clinical trials in Alzheimer's patients (AD). The Abeta protein builds up in Alzheimer's patient's brain and forms oligomers, which bind to synaptic receptors and block LTP, causing memory failure and synapse loss and ultimately dementia. Preclinical evidence indicates that sigma-2 receptors regulate the Abeta oligomer receptor complex on neurons. CT1812 allosterically modulates the sigma-2 complex, which in turn destabilizes the oligomer binding site within the oligomer receptor complex, resulting in an increased off-rate of oligomers. CT1812 treatment restores membrane and protein trafficking deficits, stops spine and synapse loss *in vitro*, and improves cognitive deficits in transgenic mouse AD models. Understanding the molecular basis of sigma-2 receptor complex interactions with Alzheimer's disease biology impacts the choice of biomarkers to monitor in CT1812 clinical trials. Recent reports in tumor cells demonstrate that sigma-2 receptor small molecule ligands bind to the protein TMEM97 (Kruse 2017), which itself binds directly to PGRMC1 (Riad 2018). Both proteins play roles in lipid metabolism and are expressed in neurons and glia *in vitro* in DIV21 hippocampal/cortical cultures. PGRMC1 plays a role in protein/lipid membrane trafficking, growth factor signaling and autophagy; reduction of PGRMC1 protein expression of 30% almost completely eliminates Abeta oligomer binding to synaptic receptors (Izzo 2014a,b). However it is not known whether TMEM97 and PGRMC1 interact directly in neurons or glia. We used the Proximity Ligation technique (DuoLink, Millipore-Sigma, St Louis, MO) to determine if these two proteins colocalize within 40 nanometers in specific cellular compartments in brain cells *in vitro*. The average PLA interaction signal intensity was 59 times higher in neuronal cell bodies than in glial cell bodies ($p<0.001$). A punctate pattern of discrete PLA interaction signal was observed along dendritic processes. This is the first report of direct interactions of these two Alzheimer's disease-relevant proteins in neurons.

Disclosures: **K.T. Sadlek:** A. Employment/Salary (full or part-time):; Cognition Therapeutics Inc. **N.J. Izzo:** A. Employment/Salary (full or part-time):; Cognition Therapeutics Inc. **C. Rehak:** A. Employment/Salary (full or part-time):; Cognition Therapeutics Inc. **R. Yurko:** A. Employment/Salary (full or part-time):; Cognition Therapeutics Inc. **L. Waybright:** A. Employment/Salary (full or part-time):; Cognition Therapeutics Inc. **E. Watto:** A. Employment/Salary (full or part-time):; Cognition Therapeutics Inc. **N. Knezovich:** A. Employment/Salary (full or part-time):; Cognition Therapeutics Inc. **K. Mozzoni:** A. Employment/Salary (full or part-time):; Cognition Therapeutics Inc. **H. Safferstein:** A. Employment/Salary (full or part-time):; Cognition Therapeutics Inc. **S.M. Catalano:** A. Employment/Salary (full or part-time):; Cognition Therapeutics Inc..

Poster

200. Small-Molecule Neurotransmitter Transport and Signaling

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 200.17/B21

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Title: Development of fluorescent small molecules and nanoprobes for the detection of the sigma-2 receptor

Authors: *C. ABATE, F. S. ABATEMATTEO, M. NISO, F. BERARDI;
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Abstract: Sigma receptors are intriguing targets implicated in diseases as diverse as tumors and neurological disorders. After their discovery in 1976, in the early 1990s the availability of a variety of ligands allowed the identification of two subtypes: sigma-1 and sigma-2. The sigma-1 subtype was cloned soon thereafter and only recently crystallized. On the other hand, the sigma-2 receptor was recently cloned and recognized as the endoplasmic reticulum resident membrane protein TMEM97 after a series of attempts for its identification. In the meantime, the high affinity and selective sigma-2 ligands that were generated during the years allowed to pharmacologically characterize the sigma-2 subtype. The receptor is overexpressed in a number of tumors and its ligands may exert antitumoral action. To these intriguing features, the involvement of the sigma-2 receptor in the Alzheimer's disease (AD) has been recently added. It was shown that sigma-2 antagonists are able to inhibit the binding of Abeta oligomers to neurons and block the Abeta toxic effects in vitro and in vivo, so that one of these compounds (Elayta, CT1812) entered clinical trials as an AD disease-modifying agent. However, a true understanding of the sigma-2 receptors physiological role remains to be achieved, and the potential medical applications in a range of diseases proper of sigma-2 receptors impose such an achievement. While the cloning of the sigma-2 receptor is a crucial step towards this direction, the availability of a variety of fluorescent probes, based on the structures of the sigma-2 highest affinity ligands known, will be helpful in the set up of studies that may lead to uncover the physiology of the sigma-2 receptor. With this perspective, we designed a series of sigma-2 fluorescent ligands and nanoprobes to be used in flow cytometry and fluorescent microscopy studies. Sigma-2 reference compounds, with diverse structural features, such as siramesine, RHM1 and PB28 were used as the pharmacophore, which was connected to fluorescent tags with diverse excitation/emission wavelengths through different linkers, with the aim of extending the use of these compounds to differently equipped instruments. Additionally, some of these pharmacophores were connected to quantum dots to obtain inorganic nanoprobes with superior fluorescent properties. Besides obtaining versatile and powerful tools for sigma-2 related fluorescence studies, the use of diverse structural cores connected to the imaging portion may

unravel questions on the cell pathways activated by the diverse sigma-2 receptor ligands, whose activity has so far appeared to be cell-dependent and ligand-dependent.

Disclosures: C. Abate: None. **F.S. Abatematteo:** None. **M. Niso:** None. **F. Berardi:** None.

Poster

200. Small-Molecule Neurotransmitter Transport and Signaling

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 200.18/B22

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Title: Autophagy regulates cocaine-induced behaviors via the Becn2 protein

Authors: Y.-J. KIM[1], S. YAMAMOTO[1], H. Y. MELTZER[2], ***C. HE**[3];
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[3]Northwestern Univ. - Chicago, Chicago, IL

Abstract: Drug abuse is one of the most important health and societal issues of our time. Cocaine is one of the most widely abused recreational drugs throughout the world, and produces a variety of behaviors, including psychostimulation, addiction, and death from overdose. The mechanisms that underlie cocaine-induced disordersare unresolved and effective treatments are lacking. We used a combination of mouse genetics, cell biology, animal behavior, and pharmacological approaches, linking cocaine psychostimulation and addiction to the autophagy pathway, a stress-induced lysosomal degradation process that breaks down damaged or unnecessary structures in the cell. Although emerging evidence indicates that autophagy proteins are implicated in several diseases, whether and how they play a role in drug abuse and addiction is essentially unknown. We discovered that an autophagy-related protein Becn2 is a novel regulator and druggable target for the prevention of cocaine-induced behaviors.Knockout of Becn2 protects mice from cocaine-induced psychostimulant and addictive effects, as well as cocaine-amplified dopamine release and signaling, due to an increase in the striatal presynaptic dopamine receptor 2 (D2R), an autoreceptor that inhibits dopamine release. We further found that Becn2 regulates D2R trafficking, degradation, and cocaine-induced behaviors via binding to an adaptor protein GASP1. In addition, translationally, we found that targeting Becn2 by upstream small-molecule autophagy inhibitors stabilizes striatal presynaptic D2R andprevents physiological and behavioral responsiveness to cocaine. Thus, these results link dopaminergic transmission to an entirely new and potentially druggable pathwayto prevent behavioral responses to cocaine, and also suggest that besides cocaine, Becn2 and the autophagy machinery may play an important role in other substance-related and/or mental disorders caused by altered dopamine transmission.

Disclosures: Y. Kim: None. **S. Yamamoto:** None. **H.Y. Meltzer:** None. **C. He:** None.

Poster

200. Small-Molecule Neurotransmitter Transport and Signaling

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 200.19/B23

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: Upjohn Professorship of Pharmacology
NIH NIGMS Pharmacology Pre-doctoral T32 Training Grant

Title: Characterization of sigma-2 receptor ligand-induced metabolic stimulation in human SK-N-SH neuroblastoma cells

Authors: B. M. MCVEIGH[1], C. Z. LIU[1], C. R. MCCURDY[2], ***W. D. BOWEN**[1];
[1]Mol. Pharmacology, Physiology, & Biotech., Brown Univ., Providence, RI; [2]Medicinal Chem., Univ. of Florida, Gainesville, FL

Abstract: The sigma-2 receptor was recently identified as TMEM97. Sigma-2 receptor agonists have traditionally been characterized as ligands that induce programmed cell death in various cell types by a variety of mechanisms. However, despite the sigma-2 pharmacological profile, a recent report shows that knock-out of TMEM97 does not affect the cytotoxic potency of some sigma-2 ligands, suggesting that sigma-2R/TMEM97 does not mediate the cytotoxic effect. We have previously reported a novel metabolically stimulative function of the sigma-2 receptor, with stimulation of glycolytic hallmarks; effects consistent with a pro-survival function and receptor upregulation in cancer cells. The effects include increased reductive capacity as indicated by stimulation of MTT reduction, increase in cellular ATP level, reduction in basal ROS level, and stabilization of HIF-1α, as determined in human SK-N-SH neuroblastoma cells. The stimulation of MTT reduction was blocked by sigma-2 receptor antagonists, supporting mediation by sigma-2 receptors. Here we further characterize this effect using additional analogs of the canonical sigma-2 antagonist, SN79. CM764, CM571, and WA504 (sigma-2 Ki = 3.5, 21.7, and 2.5 nM, respectively) all induced dose-dependent stimulation of MTT reduction. At the highest dose examined (30 µM) CM764, CM571, and WA504 induced 45%, 33%, and 75% stimulation of MTT reduction after a 24 h treatment period. An examination of the time course revealed that it takes 3 to 6 hours of treatment for this stimulative effect to fully develop. CM764 and CM571 (10 and 30 µM) were found to induce an immediate, transient, and dose-dependent increase in cytosolic calcium in Fura-2 loaded cells. Thapsigargin (150 nM) pretreatment completely eliminated the calcium response, indicating that calcium release derives from endoplasmic reticulum stores. Our previous study showed that 10 µM CM764 caused a time-dependent increase in HIF-1α level that became prominent at 6 h and continued to increase up to 24 h. HIF-1α is a global regulator of several components of the glycolytic pathway. Furthermore, there is evidence that calcium signals induce expression of HIF-1α. Therefore, it is possible that these

sigma-2 ligands impact glycolysis via immediate ER calcium release which then induces a latent downstream upregulation of HIF-1α, subsequently resulting in upregulation of glycolytic enzymes and transporters. This possiblity will be explored.

Disclosures: B.M. McVeigh: None. **C.Z. Liu:** None. **C.R. McCurdy:** None. **W.D. Bowen:** None.

Poster

200. Small-Molecule Neurotransmitter Transport and Signaling

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 200.20/B24

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Title: The sigma-2 receptor/TMEM97, PGRMC1, and LDL receptor complex are responsible for the cellular uptake of Aβ42 and its protein aggregates

Authors: *A. RIAD, Z. LENGYEL, B. JANSSEN, C. ZENG, R. H. MACH;
Radiology, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Background: Our lab has recently shown that the Sigma-2 Receptor/Transmembrane Protein 97 (TMEM97) and Progesterone Receptor Membrane Component 1 (PGRMC1) form a complex with the Low Density Lipoprotein Receptor (LDLR), and this intact complex is required for efficient uptake of lipoproteins such as LDL and apolipoprotein E (apoE). These receptors are expressed in the nervous system where they have implications in neurodegenerative diseases such as Alzheimer's disease, where apoE is involved in neuronal uptake and accumulation of Aβ42, eventually cascading into neurodegeneration, synaptic dysfunction, and ultimately, dementia.
Hypothesis: We hypothesize that the intact Sigma-2 receptor complex -TMEM97, PGRMC1, and LDLR— is necessary for internalization of apoE and monomeric, oligomeric, and fibrillated amyloid beta (Aβ42), and the disruption of the receptor complex inhibits uptake.
Methods: To study the role of these receptors in the uptake of monomeric, oligomeric, and fibrillated Aβ42 alone or when in a complex with apoE, we utilized HeLa cells as a model system and used CRISPR/Cas9 to generate TMEM97 knockout, PGRMC1 knockout, and TMEM97/PGRCM1 double knockout (DKO) cell lines. Uptake was assessed with ELISA and confocal microscopy. Treatment of control cells with the TMEM97 and PGRMC1 ligands RHM-4, SW43, and AG-205 was performed to assess the ability of small molecules to disrupt the complex and inhibiting internalization. Uptake studies using these inhibitors were also performed on primary rat cortical neurons to assess their ability to inhibit uptake in a neuronal cell culture system.
Results: The results of this study suggest that the intact Sigma-2 receptor complex is a binding site for Aβ42, and Aβ42 in complex with apoE. The loss or pharmacological inhibition of one or

both of these proteins results in the disruption of the complex leading to decreased uptake of monomeric, oligomeric, and fibrillated Aβ42.
Conclusion: The TMEM97, PGRMC1, and LDLR complex is critical for the cellular uptake of Aβ42 via apoE dependent and independent mechanisms. This study suggests that the complex may potentially be a novel pharmacological target to decrease neuronal Aβ42 internalization and accumulation, which may represent a new strategy for slowing the rate of neurotoxicity, neurodegeneration, and progression of Alzheimer's disease.

Disclosures: A. Riad: None. **Z. Lengyel:** None. **B. Janssen:** None. **C. Zeng:** None. **R.H. Mach:** None.

Poster

200. Small-Molecule Neurotransmitter Transport and Signaling

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 200.21/B25

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: NIH Grant AG055247
NIH Grant AG037337
NIH Grant AG054176

Title: Metabolomic evidence of disease modification of in mild to moderate AD patients by Sigma-2 antagonist CT1812

Authors: *N. J. IZZO[1], C. REHAK[1], K. M. MOZZONI[1], G. LOOK[1], G. RISHTON[1], L. SCHNEIDER[2], S. DEKOSKY[3], M. GRUNDMAN[1], C. HOUSER[1], S. M. CATALANO[1]; [1]Cognition Therapeut. Inc., Pittsburgh, PA; [2]USC, LOS ANGELES, CA; [3]Univ. of Florida, Gainesville, FL

Abstract: C T1812 (Elayta^{TM}) is a selective sigma-2 receptor antagonist currently in clinical trials as a disease-modifying treatment for Alzheimer's disease (AD). Amyloid-beta oligomers bind to receptors on neurons and cause synaptotoxicity and cognitive decline in Alzheimer's disease. The oligomer receptor is regulated by the sigma-2 receptor complex. When CT1812 binds sigma-2 receptors, the oligomer binding site in the receptor complex is destabilized, resulting in an increase in the off-rate of oligomers from their receptors. CT1812 rapidly displaces bound oligomers from neurons in vitro and human Alzheimer's patient brain post mortem tissue sections. Two of the hypothesized protein constituents of the sigma-2 receptor complex in neurons are TMEM97 and PGRMC1; both impact lipid metabolism, but their effects on these pathways in a clinical setting is unknown. A phase 1a/2b clinical trial of CT1812 was performed in mild to moderate AD patients (MMSE 18-26). Participants received one of three doses of CT1812 or placebo once daily for 28 days (N=19). Plasma was collected prior to first

dose and 24 hours after last dose and analyzed at the Duke University Metabolomics Center, utilizing the AbsoluteIDQ-p180 kit with analysis by UPLC/MS/MS. Comparisons were made for each patient at end of study versus their own baseline for each measured metabolite and statistical analysis was performed to determine differences between the placebo and pooled drug-treated groups. Results of this analysis revealed that 11 individual metabolites were significantly changed in CT1812-treated vs. placebo-treated patients (2-tailed Student's t test $p<0.05$). These metabolites are known to be lower in AD patients compared to age matched controls (Toledo et al 2017, Li 2016); treatment with CT1812 resulted in increases in 10 of 11 of these metabolites in each patient compared to their baseline values, consistent with a positive effect on disease course. In particular, lipid metabolites such as long chain polyunsaturated fatty acids as well as carnitines and acyl-carnitines decrease in AD patients compared to normal individuals (Toledo 2017), whereas CT1812 treatment resulted in significant increases in these metabolites compared to placebo treatment (LCPUFAs $p = 0.0276$, carnitines $p=0.0216$, ANOVA) consistent with CT1812's impact on sigma-2 receptors and downstream lipid metabolism. We conclude that in mild to moderate AD patients, 28 days of treatment with CT1812 changes many plasma metabolites in a therapeutic direction, consistent with a positive effect on disease course and its mechanism of action. Additional Phase 2 six-month trials in this patient population are currently underway.

Disclosures: **N.J. Izzo:** A. Employment/Salary (full or part-time):; Cognition Therapeutics, Inc. **C. Rehak:** A. Employment/Salary (full or part-time):; Cognition Therapeutics, Inc. **K.M. Mozzoni:** A. Employment/Salary (full or part-time):; Cognition Therapeutics, Inc. **G. Look:** A. Employment/Salary (full or part-time):; Cognition Therapeutics, Inc. **G. Rishton:** A. Employment/Salary (full or part-time):; Cognition Therapeutics, Inc. **L. Schneider:** F. Consulting Fees (e.g., advisory boards); Cognition Therapeutics, Inc. **S. Dekosky:** F. Consulting Fees (e.g., advisory boards); Cognition Therapeutics, Inc. **M. Grundman:** F. Consulting Fees (e.g., advisory boards); Cognition Therapeutics, Inc. **S.M. Catalano:** A. Employment/Salary (full or part-time):; Cognition Therapeutics, Inc. **C. Houser:** A. Employment/Salary (full or part-time):; Cognition Therapeutics, Inc..

Poster

201. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 201.01/B26

Topic: B.02. Ligand-Gated Ion Channels

Title: The novel procognitive S68890 modulates the human α7 nicotinic receptors

Authors: *L. DANOBER[1], K. KAMBARA[2], S. BERTRAND[2], K. LLOPIS[3], C. LIARD[3], G. DAS DORES[3], C. LOUIS[3], T. PILLOT[4], A. BENOIST[5], J.-M. FOURQUEZ[5], A.-M.

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Abstract: Modulation of the α7 neuronal nicotinic acetylcholine receptor (nAChRs) has become a prime target for the development of procognitive drugs aiming at counteracting deficits such as encountered in Schizophrenia or Alzheimer diseases. The finding of a small molecule the S68890 active at the human α7nAChRs opened new hopes to discover potent new compound with innovative mechanism of action. Pharmacological characterization conducted at the human recombinant receptors expressed in *Xenopus* oocytes allowed to demonstrate that S68890 exhibited much a greater selectivity for α7nAChRs than $5HT_{3A}$ receptors. Moreover, testing the S68890 across a wide panel of receptors and channels confirmed the absence of detectable cross reactivity and the excellent safety profile of this molecule. At concentrations superior to the micromolar, the S68890 inhibited in a voltage dependent manner the α7 responses to agonist suggesting that, given its small size, the S68890 might enter the α7 ionic pore and caused blockade by steric hindrance. These effects were, however, clearly distinct from concentrations efficacious in procognitive testing which are about a thousand fold lower. Experiments conducted at low concentrations of S68890 revealed that exposure in the nanomolar range enhanced up to two hundred fold the currents evoked by 40 µM ACh. Reminiscent of the effects previously reported for encenicline[1], this suggests that exposure to S68890 in the nanomolar range causes priming of the α7nAChRs. Procognitive tests using either natural-forgetting conditions but also amyloidergic-altered memories in rodents revealed that at low concentrations, S68890 displayed potent cognitive-enhancing properties. The absence of cross-reactivity of S68890 in pharmacological characterization as well as general behavior in animal testing confirms the superiority of this molecule over previously discovered compounds. Altogether, these results indicate that S68890 is a promising candidate for the treatment of cognitive impairments associated diseases.
[1]Prickaerts et al EVP-6124, a novel and selective α7 nicotinic acetylcholine receptor partial agonist, improves memory performance by potentiating the acetylcholine response of α7 nicotinic acetylcholine receptors. Neuropharmacology 2011;**62:**1099-110

Disclosures: L. Danober: None. **K. Kambara:** None. **S. Bertrand:** None. **K. Llopis:** None. **C. Liard:** None. **G. Das Dores:** None. **C. Louis:** None. **T. Pillot:** None. **A. Benoist:** None. **J. Fourquez:** None. **A. Chollet:** None. **P. Gloanec:** None. **R. Jeggo:** None. **D. Bertrand:** None.

Poster

201. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 201.02/B27

Topic: B.02. Ligand-Gated Ion Channels

Support: DA040047
DA046335

Title: Green apple tobacco flavorant farnesene is rewarding and reinforcing in a mouse model of vaping-related behavior

Authors: *S. COOPER, A. T. AKERS, A. J. AVELAR, B. J. HENDERSON;
Biomed. Res., Joan C Edwards Sch. of Med. at Marshall Univ., Huntington, WV

Abstract: Although the use of traditional cigarette smoking has been on the decline, the use of electronic nicotine delivery systems (ENDS) has increased tremendously, with over three million users being high school students. The production of ENDS products was initially intended to improve smoking cessation rates; however, it also targeted a new market of nicotine users among the adolescent population. With the increased popularity of ENDS, especially among the youth, flavor additives have become more of a concern due to the endless options available and the growing use of zero-nicotine flavored e-liquids. Yet, little is known regarding the effects of tobacco flavors on nicotine addiction and smoking-related behaviors. Previous studies have shown that menthol, the leading tobacco flavor, enhances nicotine reward and reinforcement. Similarly, we studied green apple flavor farnesene in a mouse model and observed reward-related behavior in the presence and absence of nicotine. Using a conditioned place preference assay we found that farnesene enhances nicotine reward in a sex-dependent manner while also displaying rewarding effects when administered alone. To examine vaping-related behavior, we utilized E-vape self-administration to assess the reinforcing effects of the green apple tobacco flavor with and without nicotine. Here we observed mice will self-administer green apple flavor, even in the absence of nicotine. Following behavioral assessments, we used confocal microscopy and α4-mCherryα6-GFP mice to correlate any smoking-related behaviors to potential changes in α4-containing (α4*), α6*, and α4α6* nAChRs in midbrain neurons. Farnesene treatment significantly downregulated the expression of α6*, and α4α6* nAChRs in the dopamine neurons of the ventral tegmental area, a vital region involved in the rewarding properties of addictive drugs. These farnesene-induced behavioral and cellular changes display the importance of investigation into tobacco flavors and their effect on smoking-related behaviors.

Disclosures: S. Cooper: None. **A.T. Akers:** None. **A.J. Avelar:** None. **B.J. Henderson:** None.

Poster

201. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 201.03/B28

Topic: B.02. Ligand-Gated Ion Channels

Title: Effects of autoantibodies at the human nicotinic acetylcholine receptors

Authors: *S. BERTRAND[1], K. KAMBARA[1], S. PONS[2], J. KREYE[3], N. WENKE[3], M. ZOURIDAKIS[4], S. J. TZARTOS[4,5], H. PRUESS[3], *D. BERTRAND[1], U. MASKOS[2];
[1]Hiqscreen, Geneva, Switzerland; [2]Inst. Pasteur, Paris, France; [3]AG Pruess, DZNE Berlin, Berlin, Germany; [4]Hellenic Pasteur Inst., Athens, Greece; [5]NeuroDiagnostics, Athens, Greece

Abstract: Autoimmune diseases affecting the nicotinic acetylcholine receptors (nAChRs) were first identified a long time ago with the characterization of Myastenia gravis (1), in which auto-antibodies (AAbs) are directed against the nAChRs at the neuromuscular junction. More recent studies have revealed the role of autoimmune disease for the α3β4 nAChRs expressed in the parasympathetic ganglionic system (2). However, almost nothing is known about the potential effects of AAbs that would be directed against nAChRs expressed centrally. We have characterized human monoclonal AAbs derived from cerebrospinal fluid of patients diagnosed with NMDA receptor encephalitis (3).
Taking advantage of recombinant human nAChRs expressed in *Xenopus* oocytes with functional investigations using very small volume samples, we have examined the effects of a panel of antibodies at the α7 and α4β2 nAChRs. Probing the effects of a series of AAbs, we observed a selective inhibition in the low nanomolar range caused by nine monoclonals at the α7 nAChRs. Additionally, significant activity could be detected by two of those at the heteromeric α4β2 receptors.
Altogether, these data illustrate that human AAs can interact with centrally expressed nAChRs and can be suspected, as in the case of NMDA receptor encephalitis, to be at the origin of brain pathology.

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3. J. Kreye, N. Wenke *et al.*, Human cerebrospinal fluid monoclonal N-methyl-D-aspartate receptor autoantibodies are sufficient for encephalitis pathogenesis. *Brain* 139, 2641–2652 (2016).

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Poster

201. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 201.04/B29

Topic: B.02. Ligand-Gated Ion Channels

Support: PAPIIT IN216319

Title: Mecamylamine increases the excitability of dorsal raphe serotonergic neurons

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Abstract: The dorsal raphe nucleus (DRN) is the main source of serotonin (5-HT) to the forebrain. Previous studies in our laboratory have demonstrated that nicotine increases the firing rate of 5-HT DRN neurons by promoting the release of glutamate through the stimulation of presynaptic α4-β2 nicotinic acetylcholine receptors (nAChRs) (Garduño et al., 2012). Surprisingly, other works have suggested that mecamylamine (Mec), a non-specific and non-competitive blocker of nAChRs also increases the activity of 5-HT DRN neurons and induces serotonin release (Mihailescu et al., 1997; Kenny et al., 2000; Reuben and Clark 2000). Moreover, behavioral studies have reported that Mec produces antidepressant effects in rats (Rebenstein et al., 2006). These contradictory data suggest that Mec could have other effects than blocking nAChRs in the DRN. This study was aimed to determine the mechanisms by which Mec increases the excitability of 5-HT DRN neurons. We used midbrain slices obtained from male Wistar rats (21-25 postnatal days). The electrical activity of 5-HT DRN neurons was recorded by using whole cell voltage and current clamp techniques. Besides, we performed calcium imaging experiments to record the activity of dozens of 5-HT neurons simultaneously with single cell resolution. We found that Mec (3-6 μM) increased the firing rate (≈30%) and decreased the GABAergic sIPSCs recorded from 5-HT DRN neurons. The firing frequency increase was mimicked by RJR-2403 (100nM), a highly selective agonist of α4-β2 nAChRs, and blocked by dihydro-β-eritroidine (DHβE, 100 nM) a specific antagonist of these receptors. On the other hand, the administration of PNU-28298 (100nM) a specific agonist of α7 nAChRs produced a 65% decrease of 5-HT DRN firing rate and an increase in the frequency of GABAergic sIPSCs and mIPSCs. Calcium imaging experiments showed that Mec increased the activity of the majority of DRN neurons. This increase was reversed by the bath perfusion of serotonin or the selective 5-HT1A receptor agonist, 8-OH-DPAT (5μM) suggesting that the

effect of Mec is mainly exerted on serotonergic neurons. Our data also demonstrate that Mec increases the activity of 5-HT neurons by stimulating α4-β2 nAChRs which increases glutamate release (Garduño et al., 2012) and by decreasing the inhibitory tone produced by GABA release in the DRN.

Disclosures: S. Hernandez-Lopez: None. **A. Mondragon-Garcia:** None. **O. Hernandez:** None. **E. Ramirez-Sanchez:** None. **G. Arenas-Lopez:** None. **S. Mihailescu:** None.

Poster

201. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 201.05/B30

Topic: B.02. Ligand-Gated Ion Channels

Title: Prefrontal cortex single cell dynamics and behavior during systemic nicotine exposure

Authors: J. WELLBOURNE-WOOD, *J. I. WAMSTEEKER CUSULIN, B. J. HALL; Neurosci. Discovery, Roche Innovation Ctr. Basel, F. Hoffmann-La Roche Ltd, Basel, Switzerland

Abstract: Nicotinic acetylcholine receptors (nAChRs) localized to the medial prefrontal cortex (mPFC) are hypothesized to be substrates for pro-attentional and working memory effects of nicotine. The goal of this research was to investigate the effects of nicotine on mPFC neuronal activity with single-cell resolution in freely-moving animals. In order to achieve this, we employed miniaturized microscope and GRIN-lens-based imaging techniques. GCaMP6s expression in the mPFC of male C57BL6 mice (6+ weeks postnatal) was driven by AAV viral vectors, targeted to excitatory neurons using a CaMKII promotor, while imaging lenses were chronically implanted near the injection site to visualize (layer 5-6) neurons. After recovery (6 weeks) and habituation to imaging conditions, animals were imaged during free exploration of a novel open field arena at baseline, following subcutaneous injection of nicotine (0.5 mg/kg) or vehicle, and during a washout period. After a minimum of several days, animals were retested with reversed nicotine/vehicle exposure. Behaviorally, nicotine induced a robust, but reversible hypolocomotion. Prior exposure to the arena did not significantly impact either baseline or nicotine-induced changes in locomotion. Calcium dynamics were evaluated at the level of bulk fluorescence, as well as for single cells. A range of fluorescent readouts were assessed to compare the effects of nicotine and vehicle, including frequency, amplitude, and area under the curve. Single- cell and bulk fluorescence readouts were also compared to behavioral patterns. Preliminary results indicate a relationship between bulk fluorescence and locomotor activity. Patterns of activity in bulk fluorescence were, however, unaffected by nicotine administration. At the single-cell level, the effects of nicotine could be revealed. A network effect that includes both the relative activation and deactivation of single cells compared to vehicle could be

observed, as well as an overall increase in area under the curve of single-cell fluorescence traces. These findings point to an effect of nicotine on neuronal ensemble dynamics in the mPFC. Further analyses and experiments are ongoing to assess behavioral relevance and circuit specificity of nicotine-driven network alterations.

Disclosures: **J. Wellbourne-Wood:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche Ltd. **J.I. Wamsteeker Cusulin:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche Ltd. **B.J. Hall:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche Ltd.

Poster

201. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 201.06/B31

Topic: B.02. Ligand-Gated Ion Channels

Support: NIMH Grant R01MH093354

Title: Regulatory effects of cholinergic receptor stimulation on working memory related neuronal activity in primate dorsolateral prefrontal cortex

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Abstract: The pyramidal cell circuits in dorsolateral Prefrontal cortex (dlPFC) are afflicted in Schizophrenia. Studies in primate have shown that these circuits excite each other through glutamatergic, NMDA receptor synapses on dendritic spines to generate the persistent neural representations needed for working memory, which plays a fundamental role in cognition function. Our research has shown that the stimulation of nicotinic-α7 acetylcholine receptors (α7-nAChR) enhances persistent activity of Delay cells in primate dlPFC, and is permissive for NMDAR actions, rescuing neuronal firing from NMDAR blockade. Immunoelectron microscopy data has revealed that in addition to α7-nAChR, muscarinic M1 acetylcholine receptors (M1R) are also localized in dlPFC glutamatergic-like synapses. Our new data show that M1R also plays a critical role in persistent Delay activity in primate dlPFC. Activation of M1R enhances delay-related firing within a narrow, low dose range. However, over-stimulation of either α7-nAChR or M1R impaired working memory function, potentially limiting clinical utility. We hypothesize that combined low dose stimulation of M1R and α7-nAChR will synergize to greatly enhance Delay cell firing. In this study. We iontophoretically applied low doses of the α7-nAChR agonist PHA 543613 (PHA) and the M1R positive allosteric modulator VU 0357017 (VU) to Delay cells in primate dlPFC as monkeys performed an oculomotor delayed response task. The preliminary data suggest that the same dlPFC neuron can respond to both VU or PHA and can show

synergistic enhancing effects on neuronal firing. These data suggest a potential treatment strategy to facilitate the beneficial actions of cholinergic treatments.

Disclosures: S. Yang: None. **V.C. Galvin:** None. **A.F.T. Arnsten:** None. **M. Wang:** None.

Poster

201. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 201.07/B32

Topic: B.02. Ligand-Gated Ion Channels

Title: Novel selective positive modulator efficiently decelerates decay of alpha7 nicotinic acetylcholine receptor-mediated current and increases intracellular calcium concentration in hippocampal neurons implying therapeutic potential

Authors: L. FODOR, M. THAN, I. PAL, S. KOLOK, *A. KERN, A. VISEGRADY, B. LENDVAI, Z. NEMETHY;
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Abstract: Homomeric alpha7 nicotinic acetylcholine receptor (nAChRs) is a ligand-gated pentameric ion channel expressed in the central nervous system, notably in cognition-relevant areas including the prefrontal cortex, the hippocampus and other subcortical limbic structures. Numerous studies indicate that a variety of nAChR ligands have the potential to improve multiple domains of cognition, including memory and attention. Despite extensive efforts in the nAChR field in clinical trials with orthosteric agonists, effective and durable treatments for neurocognitive disorders remain an unmet need. Therefore, we have sought and developed series of novel alpha7 nAChR selective positive modulator compounds. Modulators were selected based on their enhancing effect on intracellular Ca^{2+} responses to a selective alpha7-nAChR agonist using plate-based fluorometry and by their effect on current kinetics evoked by choline using automated patch clamp in recombinant human alpha7 nAChR-expressing cells. Selected compounds were characterized by manual patch clamp in recombinant cells and by patch clamp and multiphoton Ca^{2+} imaging in interneurons from rat hippocampal slices. Compounds varied broadly in terms of their effects on current kinetics as well as positive modulatory and agonist potencies. A representative compound was selected to display the decelerating effect on the decay of choline-induced currents from 100 nM without agonistic effect. From 1 μM it evoked an inward current and showed pronounced current decay-decelerating effect with rapid onset and offset. This compound elicited a significant increase in choline-evoked responses for both potency and efficacy. Using multiphoton microscopy, it significantly enhanced choline-evoked intracellular Ca^{2+} elevation at the site of choline injection in the dendritic processes of hippocampal interneurons. Both electrophysiology and $[Ca^{2+}]_i$ imaging data indicated compound-induced enhancement of spontaneous activity of hippocampal interneurons. All these

effects could be blocked by the alpha7 nAChR-selective antagonist methyllycaconitine (MLA). The above in vitro data uncover unique and promising properties of this novel alpha7 nAChR positive modulator and deepen our understanding of nicotinic actions at the receptor level. Further development of these compounds may provide an efficient strategy for treatment of cognitive disorders.

Disclosures: L. Fodor: None. **M. Than:** None. **I. Pal:** None. **S. Kolok:** None. **A. Kern:** None. **A. Visegrady:** None. **B. Lendvai:** None. **Z. Nemethy:** None.

Poster

201. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 201.08/B33

Topic: B.02. Ligand-Gated Ion Channels

Support: CIHR MOP 89825

Title: Maturational changes in the recruitment of prefrontal layer 6 neurons by acetylcholine

Authors: *T. CHEN[1], S. VENKATESAN[1], Y. LIU[1], E. E. TURNER[2], E. K. LAMBE[1]; [1]Dept. of Physiol., Univ. of Toronto, Toronto, ON, Canada; [2]Ctr. for Integrative Brain Res., Seattle Children's Res. Inst., Seattle, WA

Abstract: Corticothalamic neurons in layer 6 of the medial prefrontal cortex (PFC) participate in attention, but this aspect of cognition is known to mature relatively late in development. Does this pattern of delayed development also hold true for the cholinergic modulation of layer 6 neurons which has been implicated in controlling attention? In adulthood, layer 6 neurons are modulated by acetylcholine through activation of nicotinic and muscarinic cholinergic receptors, but how the contributions of these receptors interact during postnatal development is not well understood. To survey the layer 6 neurons in medial prefrontal cortex, we used a BAC transgenic mouse line capable of expressing a genetically-encoded calcium indicator in this population (Syt6-Cre:GCaMP6s). In acute brain slices, we used multiphoton microscopy to detect increases in intracellular Ca2+ that occur when acetylcholine excites layer 6 neurons to fire a train of action potentials. Since there are prominent changes in the expression of nicotinic and muscarinic receptors across postnatal development, to date we have examined male and female mice from the juvenile period to adulthood. Different concentrations of exogenous acetylcholine were applied to test concentration response, and cholinergic receptor blockers were applied to test receptor specificity. We found that in juvenile mice, layer 6 neurons showed a greater response to lower concentrations of acetylcholine than the adult mice. Pharmacological examination showed that, while there were prominent nicotinic and muscarinic receptor-mediated components to the layer 6 cholinergic response across the postnatal stages tested, the

left-shifted concentration response curve was attributable to a nicotinic mechanism. To investigate the role of an early-expressed but pharmacologically-intractable subunit of the nicotinic receptor in this early postnatal cholinergic sensitivity, we have created a compound transgenic mouse with Syt6-Cre:GCaMP6s expression together with a global deletion of α5 as well as littermate controls for examination across a range of developmental stages. This work suggests that cholinergic modulation may shape the maturation of prefrontal executive circuitry as well as modulating it in adulthood.

Disclosures: **T. Chen:** None. **S. Venkatesan:** None. **Y. Liu:** None. **E.E. Turner:** None. **E.K. Lambe:** None.

Poster

201. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 201.09/B34

Topic: B.02. Ligand-Gated Ion Channels

Support: R01 AA014445
R01 AA023999
P50 AA026117
R21 AA026572

Title: Investigating the role of α_7 nicotinic acetylcholine receptors (nAChRs) in the formation of alcohol-withdrawal induced anxiety within the basolateral amygdala (BLA)

Authors: *S. E. SIZER[1], B. C. PARRISH[1], N. J. ALEXANDER[1], A. B. KOUCHEKI[1], B. A. MCCOOL[2];
[1]Wake Forest Univ., Winston Salem, NC; [2]Physiol. & Pharmacol., Wake Forest Sch. of Med., Winston Salem, NC

Abstract: The lateral/basolateral amygdala (BLA) is a brain region involved in the progression of alcohol-withdrawal induced anxiety. Nucelus basalis magnocellularis (NBM) cholinergic projections to the basolateral amygdala (BLA) modulate neuronal activity and are involved in fear conditioning, memory formation, and synaptic plasticity (Jiang et al., 2016; Aitta-aho et al., 2018). Nicotinic acetylcholine receptors (nAChRs), especially α_7 nAChRs, are intimately involved in these processes. Located presynaptically on glutamatergic terminals and GABAergic interneurons, α_7 nAChRs facilitate GABA and glutamate release. Previous laboratory publications suggest that alterations in GABA and glutamate release occur following chronic intermittent ethanol (CIE) exposure. Three days of CIE exposure significantly decreases the paired-pulse ratios in BLA pyramidal neurons; this effect is indicative of an increase in glutamate release probability onto BLA pyramidal neurons (Morales et al. 2018). In the present

study, we sought to determine whether alterations in α_7 nAChR activity contributes to CIE-induced synaptic pathology during alcohol withdrawal. Male Sprague-Dawley rats were exposed to CIE in vapor chambers or ambient air for 10 days. Following 24 hours of withdrawal, rats were sacrificed for electrophysiological studies. We recorded electrically stimulated paired-pulse ratios as a measure of presynaptic function in AIR and CIE animals. We found that application of PNU-292987 and MLA (α_7 nAChR agonist and antagonist, respectively) differentially affected the release probability of AIR and CIE animals. Application of PNU-282987 significantly decreased the paired-pulse ratio in AIR, but not CIE animals. Conversely, application of MLA significantly increased the paired-pulse ratio of CIE animals to control levels, and caused no significant change in AIR animals. Taken together this data suggests that α_7 nAChR activity may be upregulated during alcohol withdrawal. This effect might be a consequence of increased acetylcholine release from NBM terminals following CIE exposure.

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Poster

201. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 201.10/B35

Topic: B.02. Ligand-Gated Ion Channels

Support: NIDA R01 DA043567
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Sigma Xi GIAR

Title: Defining the critical role of lynx2-mediated regulation of nAChRs in the basolateral amygdala for complex anxiety- and fear-related behaviors

Authors: *K. R. ANDERSON[1], K. M. HOFFMAN[1], J. M. MIWA[2];
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Abstract: Anxiety, in response to stressors, is thought to be advantageous but both erroneous or overgeneralized stress assessment and the continuation of anxiety past the stressor can lead to negative consequences. Not all stress-exposed individuals experience negative outcomes, but some do develop disorders, suggesting underlying factors can influence the ability to navigate stressors. Individual differences in biological factors such as genetics and the efficacy of neurotransmitter systems can act to modulate responses and predispose some to anxiety disorder development.The cholinergic system, a widespread modulatory neurotransmitter system, has been highly implicated in the regulation of anxiety- and fear-related behaviors. Specific nicotinic

acetylcholine receptor (nAChR) subtypes of the cholinergic system have been implicated in regulating the network excitability of the amygdala, the brain region widely implicated as the mediator of the emotional output of fear and anxiety phenotypes across species. Cholinergic modulation, therefore, is a mechanism for the investigation of anxiolytic strategies. Regulation of cholinergic signaling can be influenced by genetic factors. The lynx2 gene, which encodes a negative nicotinic protein modulator, affects anxiety and fear circuits. Mice lacking lynx2, lynx2KO mice, display heightened anxiety-like behaviors across assays and increased neuronal excitability. We hypothesize that lynx2 modulation of nAChRs within the amygdala acts to regulate responses to salient behavioral paradigms. We predict the loss of lynx2 and subsequent nAChR hyperactivity will lead to the development of altered anxiety-like and fear-like responses in the fear extinction paradigm and in response to chronic social defeat stress. To address this, we are pairing site specific focal replacement and knockdown studies with electrophysiology to study these behaviors and potential underlying mechanisms. Functional comparative studies are in progress to understand how the knowledge of lynx2 functioning in the mouse can be applied to humans. We hypothesize further investigation into lynx2 and cholinergic pathway modulation can aid in understanding the biological basis of divergent stress responses.

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

Poster

201. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 201.11/B36

Topic: B.02. Ligand-Gated Ion Channels

Title: Pathogenic beta amyloid 1-42 alters intracellular signaling through the alpha7 nicotinic receptor

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Abstract: Alzheimer's disease (AD) is a neurodegenerative disease inflicting more than 5.8 million people in the United States leading to a growing public health crisis that burdens individuals, families, and society. Current pharmacological treatments for AD center on prolonging activity of cholinergic mechanisms that underlie cognitive and memory impairments in AD. Ongoing efforts to develop better medications are stymied by a gap in understanding how the disease pathophysiology impacts cholinergic (and other forms) of neural signaling. Based on

previous findings that indicate an interaction between the α7 nicotinic acetylcholine receptor (α7 nAChR) and various amyloid proteins, we examined the ability of beta amyloid Aβ1-42 at 100nM and 500nM concentrations to modify α7 nAChR signaling in cultured neural cells. Specifically, we measured changes in α7 nAChR-mediated cytoskeletal growth, intracellular calcium release, and G-protein associated downstream signaling in the presence of Aβ1-42 and a smaller derivative peptide, Aβcore, which contains amino acids 10-15 of the protein. Our findings suggest an effect of both molecules on local calcium increases and changes in neurite growth which may be related to changes noticed in surface expression of the α7 nAChR and point to a role for Aβ1-42 in regulating aspects of α7 nAChR intracellular signaling.

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Poster

201. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 201.12/B37

Topic: B.02. Ligand-Gated Ion Channels

Title: Alpha7 nAChRs modulation of astrocytic calcium responses in acute hippocampal slices in wildtype and a 15q13.3 microdeletion mouse model using the genetically encoded calcium indicator GCaMP6f

Authors: K. A. REES[1], ***U. H. WINZER-SERHAN**[2];
[1]Dept. Neurosci. & Exp. Therap., Texas A&M Univ. Hlth. Sci. Ctr., Bryan, TX; [2]Texas A&M Hlth. Sci. Ctr., Bryan, TX

Abstract: The 15q13.3 microdeletion (MD) syndrome consists of a variable phenotype, but is most commonly associated with intellectual disability, epilepsy, schizophrenia, and autism. The 15q13.3MD consists of six genes: CHRNA7, OTUD7A, FAN1, MTMR10, TRPM1, and KLF13. CHRNA7, which codes for the α7 nicotinic acetylcholine receptor (α7 nAChR) subunit, is considered a potential driver gene for the behavioral manifestations. A translationally relevant mouse model with a homologous deletion on mouse chromosome 7qC, Df(h15q13)/+, was created, and displays behavioral features of the 15q13.3MD syndrome. The α7 nAChR assembles as homomeric cation channels with high calcium permeability. Studies have shown functional expression of α7 nAChRs on hippocampal astrocytes where they regulate astrocytic release of d-serine. In this study, we used young adult male wild-type (WT), heterozygous (HT, Df(h15q13)/+), and homozygous (KO, Df(h15q13)/-) mice to determine α7 nAChR mediated astrocytic responses, and if calcium dynamics of astrocytes were altered between genotypes. To visualize the intracellular calcium activity, an AAV5 viral vector containing the genetically encoded calcium indicator, GCaMP6f with an astrocyte specific promotor (GfaABC1D), was injected into the dorsal hippocampus. Two weeks after injection, hippocampal astrocytes in the

CA1 stratum radiatum from acute brain slices were imaged, and the somatic calcium transients were quantified. Spontaneous calcium oscillations were observed in the soma of astrocytes without any genotypic difference. The majority of astrocytes showed a significant increase over basal calcium activity in response to bath application of the α7 specific agonist choline (10 mM) (n= 31 cells, p=0.01), and further enhancement with co-application of the α7 specific positive allosteric modulator, PNU-120596 (5 μM) (n=37 cells, p=0.001) (Choline vs Choline +PNU: p=0.002). Effects of choline+PNU were blocked by the α7 nAChR antagonist, MLA. There was a significant genotype-drug interaction with choline+PNU application (p=0.006), with the positive response in the WT compared to the absence of a response in the KO (p=0.019). While HT mice were not significantly different from either WT or KO mice, the average change in the calcium dynamics in HT mice to choline+PNU was decreased by ~37% compared to responses in WT. In conclusion, we were able to detect α7 nAChR mediated responses in astrocytes from WT and HT but not KO mice, along with significant genotype-drug interactions between WT, HT, and KO Df(h15q13) mice. Thus, altered α7 nAChR-mediated astrocytic responses may contribute to neurological deficits associated with the 15q13.3. MD.

Disclosures: **K.A. Rees:** None. **U.H. Winzer-Serhan:** None.

Poster

201. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 201.13/B38

Topic: B.02. Ligand-Gated Ion Channels

Support: CIHR MOP 89825

Title: Calcium imaging in Chrna5-positive neurons of the interpeduncular nucleus across postnatal development

Authors: *Y. K. LIU[1], S. SIVAKUMARAN[1], T. CHEN[1], E. E. TURNER[2], E. K. LAMBE[1]; [1]Dept. of Physiol., Univ. of Toronto, Toronto, ON, Canada; [2]Ctr. for Integrative Brain Res., Seattle Children's Res. Inst., Seattle, WA

Abstract: The interpeduncular nucleus has been linked to nicotine aversion after exposure, a phenomenon potentially protective against further nicotine use. This region is the target of cholinergic neurons in the ventromedial habenula, and interpeduncular neurons express both nicotinic and muscarinic acetylcholine receptors (Arvin et al., 2019). Of note, the interpeduncular nucleus is the site of highest expression of the α5 nicotinic receptor subunit encoded by the *Chrna5* gene aversion and its neurons participate in the formation of aversive connections in a nicotine-sensitive manner (Morton et al., 2018). A transgenic mouse has recently been created to permit the selective labelling of α5-containing interpeduncular neurons

with Cre-recombinase sensitive reporters (Morton et al., 2018). In *Chrna5*cre/Ai96 mice, we examine the cholinergic response of these α5-containing interpeduncular neurons as a population with multiphoton imaging in acute interpeduncular brain slices. This approach allows the investigation of neuronal activity in the interpeduncular nucleus from mice without prior surgical interventions *in vivo*. This permits the examination of the pharmacological profile of supra-threshold excitatory responses within α5-positive neurons in both sexes across a range of postnatal developmental ages from juvenile to adulthood. Strong and coherent stimulation of this population is seen in response to cholinergic stimulation, yet the dose response profile changes across development. Given the heterogeneity of nicotinic receptor subunits expressed in the interpeduncular nucleus, we were surprised to observe the sensitivity of the excitatory responses across the population of *Chrna5*-positive neurons by dihydro-β-erythroidine (DHβE)-mediated blockade of β2-containing nicotinic receptors. Ongoing experiments are probing further the pharmacological modulation of *Chrna5*-positive interpeduncular neurons with the goal of understanding developmental changes in the sensitivity of this population in nicotine-naïve subjects.

Disclosures: Y.K. Liu: None. **S. Sivakumaran:** None. **T. Chen:** None. **E.E. Turner:** None. **E.K. Lambe:** None.

Poster

201. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 201.14/B39

Topic: B.02. Ligand-Gated Ion Channels

Support: CIHR MOP 89825

Title: Cholinergic signalling dysregulation in the prefrontal cortex of the TgF344 rat model of Alzheimer's disease

Authors: *S. K. POWER[1], S. VENKATESAN[1], J. MCNABB[1], J. MCLAURIN[5,2], E. K. LAMBE[1,3,4];
[1]Physiol., [2]Lab. Med. and Pathobiology, [3]Obstetrics and Gynaecology, [4]Psychiatry, Univ. of Toronto, Toronto, ON, Canada; [5]Biol. Sci. and Hurvitz Brain Sci. Res. Program, Sunnybrook Res. Inst., Toronto, ON, Canada

Abstract: “If you don’t attend, you can’t encode.” (Romberg et al., 2013) Disruption of attention and deficits in executive function occur early in Alzheimer’s disease (AD) pathology and can contribute to the decline of memory and other cognitive processes. Improving the early disruption of attention in AD is a potentially high-impact treatment goal. Cholinergic signalling in the prefrontal cortex is vital for attentional control and executive function, yet much remains

unknown about the cellular mechanisms of attentional dysfunction in early to mid-disease AD. Here, we investigate the TgF344 rat model of AD. In comparison to previous rodent models of AD, this model more closely recapitulates the molecular and behavioural trajectory of human AD. To understand the underlying changes occurring in prefrontal circuits, we use whole cell electrophysiology in acute brain slices to probe the cell properties and cholinergic modulation of the pyramidal neurons that are the major output neurons of the prefrontal cortex. For perspective, TgF344 AD rats start to exhibit deficits in executive function after 9 months of age relative to non-transgenic littermates. In electrophysiological experiments at the equivalent of mid-stage disease (18 months), there do not appear to be effects of genotype on the intrinsic cellular properties of prefrontal pyramidal neurons. Yet, there is a striking difference in the effect of cholinergic stimulation on the excitation of these pyramidal neurons. This genotype difference appears most profound in layer V, but is also evident in layer VI. The TgAD rat neurons show less precise timing and greater heterogeneity in their cholinergic excitation, with a greater proportion of neurons failing to respond and others showing delayed and/or extended excitation. Ongoing experiments are probing the time course of the emergence of the dysfunction(s) relative to the onset of the behavioural symptoms and investigating the cholinergic receptor pharmacology and the underlying cellular and circuit mechanisms. We hope these findings will improve our understanding of how attention is disrupted in AD and provide insight into potential treatment targets to ameliorate prefrontal cholinergic dysfunction in a high-fidelity model of human AD.

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Poster

201. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 201.15/B40

Topic: B.04. Ion Channels

Title: Cholinergic modulation of ionic currents and a novel oscillatory activity in human fetal cholinergic neurons from the nucleus basalis of Meynert

Authors: *F. CHERCHI[1], E. COPPI[1], I. FUSCO[1], E. SARCHIELLI[2], G. GUARNIERI[2], G. VANNELLI[2], F. PEDATA[1], A. MORELLI[2], A. M. PUGLIESE[1];
[1]Neuroscience, Psychology, Drug Res. and Child Hlth., [2]Exptl. and Clin. Med., Univ. of Florence, Florence, Italy

Abstract: The degeneration of cholinergic neurons in the Nucleus Basalis of Meynert (NBM) is responsible for the gradual cognitive decline in Alzheimer's disease (AD). To date no resolutive therapies exist. Understanding the mechanisms driving human neuroblast differentiation towards

the cholinergic phenotype could help identifying efficient therapies aimed at preventing neuronal loss in neurodegenerative pathologies, including AD. We recently characterized a primary culture (p4-p25) of human fetal NBM cells (hfNBM), isolated from 12-week old fetuses (Morelli et al., 2017, Front Cell Neurosci 11:339), as a population of cholinergic neurons expressing muscarinic and nicotinic receptors, as well as enzymes essential for acetylcholine (Ach) synthesis, degradation and vesicular transporter. By patch-clamp experiments, we report here two different functional responses, induced by Ach in these cells. A first effect consisted in an Ach (0.1-100 µM)-dependent increase in voltage-dependent K^+ currents evoked by a voltage ramp protocol (from -120 to +150mV; 1 s). This effect was mimicked by carbachol (Cch: 0.1-100 µM), prevented by the muscarinic antagonist atropine (atr: 100 nM), by the K^+ channel blocker TEA (3 mM) and by pertussis toxin pre-incubation (PTX: 1 µg/ml), demonstrating the involvement of G_i-protein-coupled M2/M4 receptors effects. The second effect induced by Ach (0.1-100 µM) was an atropine-sensitive decrease of voltage-gated Na^+ currents. This effect was mimicked by Cch and prevented by intracellular neomycin (500 µM), a phospholipase C inhibitor, demonstrating the involvement of M1/M3/M5 muscarinic receptors. Finally, when recorded in the current-clamp mode, these cells exhibited a novel, high frequency (50-80 Hz), oscillatory activity evoked by positive current injection (500-800 pA). This activity was insensitive to TTX (1-30 µM), Cd^{2+} (1 mM) or cholinergic ligands but was impaired by intracellular BAPTA (10 mM) or tapsigargin (1 µM) and blocked by extracellular TEA (200 µM), iberiotoxin (200 nM), a selective BK channel blocker, and by Ba^{2+} (2 mM), indicating the involvement of intracellular Ca^{2+} and of BK and Kir channel opening. This study provides the first description of a novel, high frequency and Ca^{2+}-dependent, voltage activity in human neuroblasts and adds novel insight into the comprehension of cholinergic modulation of NBM neurons during development. These notions could provide a useful tool to assess disease modeling and drug screening in neurodegenerative disorders.

Disclosures: **F. Cherchi:** None. **E. Coppi:** None. **I. Fusco:** None. **E. Sarchielli:** None. **G. Guarnieri:** None. **G. Vannelli:** None. **F. Pedata:** None. **A. Morelli:** None. **A.M. Pugliese:** None.

Poster

201. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 201.16/B41

Topic: B.02. Ligand-Gated Ion Channels

Support: CIHR MOP 89825

Title: Influence of Chrna5 on signaling properties of the habenulopeduncular pathway

Authors: *S. SIVAKUMARAN[1], Y. LIU[1], T. CHEN[1], D. W. SPARKS[1], E. E. TURNER[2], E. K. LAMBE[1];
[1]Physiol., Univ. of Toronto, Toronto, ON, Canada; [2]Ctr. for Integrative Brain Res., Seattle Children's Res. Inst., Seattle, WA

Abstract: The midbrain habenulopeduncular pathway has been implicated in the development of aversion and appears particularly relevant for nicotine aversion (Morton et al., 2018), but the neural mechanisms of this phenomenon remain unclear. This pathway involves cholinergic neurons in the ventral medial habenula (vMHb) innervating interneurons in the interpeduncular (IP) nucleus. The IP presents a remarkably diverse set of nicotinic acetylcholine receptor (nAChR) subunits; including the strongest expression of the α5 nAChR subunit in the mouse brain. This subunit is encoded by the *Chrna5* gene, in which a genetic polymorphism has been linked in both human and mice to decreased nicotine aversion. Yet, the habenulopeduncular pathway is not exclusively cholinergic, its vMHb neurons express *VGluT2* and use glutamate as their primary fast neurotransmitter (Ren et al., 2011). Here, we use whole cell electrophysiology and optogenetics in acute brain slices to analyze neurotransmission from the vMHb onto interneurons in the IPN of adult ChATChR2 mice either expressing (α5WT) or deleted for *Chrna5* (α5KO). We find that optogenetic activation of habenulopeduncular afferents induces excitatory postsynaptic currents (EPSCs) in the majority of rostral IP neurons from both genotypes. In ongoing work, we are examining responses to 5 second trains of optogenetic stimuli, with 10-20 Hz stimulus frequencies selected to simulate peak firing seen in vMHb neurons in response to acute stimulation (Morton et al., 2018) and higher stimulus frequencies thought to preferentially elicit cholinergic release (Zhao et al., 2011; Ren et al., 2011). Short-term changes in the frequency and amplitude of evoked excitatory postsynaptic currents (evEPSCs) are seen across the 10-20 Hz stimulation trains, with initial depression followed by facilitation, and these EPSCs are suppressed by the glutamatergic antagonist DNQX. The 50 Hz trains elicit slow-rising, prolonged and repeatable inward currents, which differ from currents evoked by nicotine or acetylcholine in their insensitivity to DHBE. We have created an additional compound transgenic mouse to examine the optogenetic fast evEPSC and slow inward current responses in brain slices with labeled *Chrna5*-positive neurons.

Disclosures: S. Sivakumaran: None. **Y. Liu:** None. **T. Chen:** None. **D.W. Sparks:** None. **E.E. Turner:** None. **E.K. Lambe:** None.

Poster

201. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 201.17/B42

Topic: B.02. Ligand-Gated Ion Channels

Support: CIHR MOP 89825

Title: Prefrontal alpha5 nicotinic receptor: Essential for a rapid cholinergic response and its protection from nicotine

Authors: *S. VENKATESAN[1], E. E. TURNER[2], E. K. LAMBE[1];
[1]Dept. of Physiol., Univ. of Toronto, Toronto, ON, Canada; [2]Ctr. for Integrative Brain Res., Seattle Children's Res. Inst., Seattle, WA

Abstract: Cholinergic neuromodulation of layer 6 corticothalamic neurons in the prefrontal cortex (PFC) is important for top-down control of attention. Layer 6 pyramidal neurons in the PFC express the α5 nicotinic acetylcholine (ACh) receptor subunit encoded by *Chrna5* which is critical for performing demanding attention tasks. Here, RNAscope demonstrates a substantial population of layer 6 neurons in prelimbic and cingulate cortex express α5.Yet the cellular mechanisms by which the α5 subunit influences attention remain unclear. To investigate these mechanisms in the context of endogenous acetylcholine signaling, we use optogenetic release of acetylcholine in brain slices and measure cholinergic responses in layer 6 PFC neurons in WT and $\alpha5^{-/-}$ mice. Both male and female mice were used, and no obvious sex differences were observed in the cholinergic responses to trains of light stimuli. Our results show that neurons lacking the α5 subunit show light-evoked cholinergic responses that are of similar magnitude to the WT neurons but are slower to respond $p<0.05$) and take longer to decay ($p<0.01$, unpaired t-test). This pattern suggests that there may be genotype differences in the localization of nicotinic receptors relative to their presynaptic release sites. Consistent with this hypothesis, encouraging spillover of light-evoked acetylcholine by blocking acetylcholinesterase with DFP significantly increased peak responses in the WT. Yet there was no detectable increase in the peak response in the $\alpha5^{-/-}$, raising a question about the response potentially being limited by desensitization. To test this last hypothesis more directly, we examined light-evoked cholinergic responses before and during application of a low concentration of nicotine. Responses in WT neurons were resistant to application of 100 nM nicotine, whereas, in the $\alpha5^{-/-}$, nicotine abolished the cholinergic responses due to desensitization of nicotinic receptors lacking the α5 subunit. Taken together, these findings point to an important role for the α5 nicotinic receptor subunit in allowing a rapid cholinergic response but also in allowing this response to be sustained when challenging or stressful conditions greatly elevate prefrontal acetylcholine levels.

Disclosures: S. Venkatesan: None. **E.E. Turner:** None. **E.K. Lambe:** None.

Poster

201. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 201.18/B43

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH grant ES027822

Title: Inhibition of α7 nicotinic receptors by R,S-Trihexyphenidyl: Relevance for treatment of organophosphorus intoxication

Authors: *Y. ARACAVA, E. X. ALBUQUERQUE, E. F. R. PEREIRA;
Div. of Translational Toxicology, Dept. Epidemiology and Publ. Hlth., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Acute poisoning with organophosphorus (OP) insecticides can be a life-threatening condition and is treated with high doses of atropine to block muscarinic receptor (mAChR) overactivation by acetylcholine (ACh) that accumulates due to OP-induced irreversible inhibition of acetylcholinesterase (AChE). Yet, despite therapeutic intervention, poor health outcomes have been reported among OP-poisoned patients, including pregnant women. Earlier studies have shown that *R,S*-trihexyphenidyl (THP), a drug approved for treatment of Parkinson's disease and dystonia, is more effective than the non-selective mAChR antagonist atropine in suppressing acute OP poisoning in adult rodents (Epilepsy Res 38:1-14, 2000). We are currently comparing the effectiveness of atropine and THP to treat acute OP intoxication during pregnancy in guinea pigs. In contrast to atropine, THP more selectively inhibits M1 and M3 than M2 mAChRs. By sparing presynaptic M2 mAChRs, THP can safeguard a negative feedback mechanism in which ACh limits its own release. However, it has also been reported that THP inhibits as-of-yet unidentified subtypes of neuronal nicotinic ACh receptors (nAChRs). Here, the whole-cell mode of the patch-clamp technique was applied to hippocampal neurons to define the pharmacological profile of THP on well-defined nAChR subtypes. THP (1-50 μM), time and concentration dependently, inhibited rapidly decaying whole-cell currents evoked by the α7 nAChR agonist choline (10 mM) in interneurons of the *stratum radiatum* (SR) of the CA1 field of the guinea pig hippocampus. By contrast, non-α7 nAChR currents evoked by 1 mM ACh in CA1 SR interneurons were found to be insensitive to inhibition by as much as 50 μM THP. This is the first demonstration that THP inhibits α7 nAChRs in hippocampal neurons. Experiments are currently underway to determine the mechanism of action of THP on α7 nAChRs and the potential involvement of these receptors on THP's ability to counter the neurotoxic effects of the OP insecticide chlorpyrifos (CPF) in primary hippocampal cultures. Inhibition of specific nAChRs by THP may be an important determinant of its effectiveness as a therapeutic intervention to counter the acute toxicity of CPF and other OP insecticides.

Disclosures: Y. Aracava: None. **E.X. Albuquerque:** None. **E.F.R. Pereira:** None.

Poster

201. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 201.19/B44

Topic: B.07. Synaptic Plasticity

Support: DA035430
DA044760

Title: Nicotine-induced golgi fragmentation in cultured cells and neurons expressing α4β2-type nicotinic receptors

Authors: *A. P. GOVIND[1], O. JEYIFOUS[1], L. NEWELL[1], A. V. WEIGEL[2], J. LIPPINCOTT-SCHWARTZ[2], W. N. GREEN[1];
[1]Dept. of Neurobio., Univ. of Chicago, Chicago, IL; [2]Janelia Res. Campus, Ashburn, VA

Abstract: Nicotine exposure produces long-lasting changes at synapses that remodel neural circuits, causing addiction. The mechanisms underlying this process remain poorly understood. Nicotine exposure causes upregulation of nicotinic acetylcholine receptors in the brain. Here, we report that long-term nicotine exposure altered the secretory pathway of α4β2-type nicotinic acetylcholine receptors (α4β2Rs) by dispersing and redistributing the Golgi apparatus. Using fluorescently tagged Golgi resident proteins we demonstrate the dose-dependent effect of nicotine on Golgi dispersal in HEK cells stably expressing α4β2Rs. Characterization *in vitro,* demonstrated that dispersal was reversible and that fragmented Golgi membranes were functional. The onset and reversal of nicotine-induced Golgi dispersal coincides with our previous measurements on the time course of nicotine upregulation of α4β2Rs. The dispersed Golgi fragments were heterogenous in size with smaller vesicles originating from larger "ministacks" similar to dispersal by Nocadazole. However, unlike Nocadozole, nicotine had no effects on microtubule distribution and acts through a different mechanism. Larger Golgi ministacks contained both cis/medial and trans Golgi proteins whereas the smaller fragments and only contained trans-Golgi glycan modifying enzymes. Similar effects of nicotine were only observed in cultured neurons expressing α4β2Rs, and the dispersed Golgi membranes were observed in somata without nicotine exposure. The smaller fragments lacking cis-Golgi markers were widely distributed throughout dendrites while larger fragments containing cis-Golgi markers (Golgi outposts) were sparsely distributed. A significant increase (2-fold) in small Golgi vesicle density was observed in the dendrites with nicotine exposure. Our results reveal that Golgi dispersal and its distribution throughout dendrites is nicotine-dependent resulting in regulated placement of membranes that can function in secretion at local subdomains.

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Poster

201. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 201.20/B45

Topic: B.07. Synaptic Plasticity

Support: NIH Grant DA035430
NIH Grant DA044760

Title: Nicotine exposure alters the distribution of dispersed Golgi membranes in axons *in vitro* and *in vivo*

Authors: *O. B. JEYIFOUS, A. P. GOVIND, L. O. VAASJO, J. L. KORANDA, X. ZHUANG, W. N. GREEN;
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Abstract: The canonical Golgi elements that mediate the last steps in the processing of secreted and membrane proteins have not been observed in axons. Here, we report on the observation and initial characterization of a dispersed Golgi compartment in axons, whose distribution and number can be regulated by exposure to nicotine. We used fluorescently-tagged, Golgi marker proteins, and antibodies (Abs) to immunostain for their endogenous counterparts, to test for dispersed Golgi membranes in the axons of cultured neurons. Golgi membranes were observed throughout most axons. Larger, dispersed Golgi elements that contained cis-, medial-, and trans-Golgi markers found in somata and dendrites were not observed in axons. Only smaller sized fragments containing trans-Golgi markers were observed, and at a lower density than in dendrites (~3-fold less). In cultured neurons expressing α4β2-type nicotinic acetylcholine receptors (α4β2Rs), a ~2-fold increase in the axonal density of these small Golgi structures was observed. Similar changes were observed *in vivo,* in mice exposed to nicotine in the drinking water. Brain sections from midbrain were stained with tyrosine hydroxylase-specific Abs to identify ventral tegmental area (VTA) dopaminergic terminals in the mouse nucleus accumbens, which are known to express α4β2Rs. Co-staining the sections with trans-Golgi markers validated in cultured neurons, we observed dispersed Golgi membranes in the VTA dopaminergic terminals with a density similar to that of the axons of cultured neurons. We observed an almost 2-fold increase in the axonal density of the Golgi membranes when nicotine was added to the drinking water. Our findings reveal a nicotine-regulated Golgi compartment in axons that appears to function in secretion at local subdomains and contribute to the molecular mechanisms underlying drug-induced plasticity.

Disclosures: O.B. Jeyifous: None. **A.P. Govind:** None. **L.O. Vaasjo:** None. **J.L. Koranda:** None. **X. Zhuang:** None. **W.N. Green:** None.

Poster

201. Nicotinic Acetylcholine Receptors: Physiology and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 201.21/B46

Topic: B.07. Synaptic Plasticity

Support: NIH Grant 5R01DA044760-02
NIH Grant T32DA043469

Title: Changes in synaptic activity in neurons regulate golgi membrane dispersal and distribution

Authors: *T. A. RUSSELL, A. P. GOVIND, O. JEYIFOUS, W. N. GREEN;
Neurobio., Univ. of Chicago, Chicago, IL

Abstract: We have found that long-term nicotine exposure both *in vivo* and *in vitro* induces a dispersal and redistribution of the Golgi apparatus throughout somata, dendrites and axons, but only in neurons expressing α4β2-type nicotinic acetylcholine receptors (α4β2Rs). In cultured neurons, we find that Golgi dispersal occurs in the absence of nicotine exposure and α4β2R expression, and the degree of Golgi dispersal is variable. In a previous study, the degree of Golgi dispersal in the soma was demonstrated to be a function of the levels of synaptic activity in the dendrites (Thayer et al., *PNAS*, 2013). Therefore, we tested whether similar treatments altered Golgi dispersal in dendrites and axons of cultured neurons. Specifically, we treated with the GABA-A receptor antagonist bicuculline and examined the number and distribution of vesicles containing the trans-Golgi marker proteins. Bicuculline treatment lead to a 1.5-fold increase in Golgi vesicles in dendrites and a 2-fold increase in axons of pyramidal neurons compared to vehicle treatment. Additionally, NMDA receptor activation via APV withdrawal led to a similar effect compared to vehicle or chronic APV treatment. Our results demonstrate that increasing synaptic activity in dendrites regulates the process of Golgi dispersal in neurons and increases the trafficking and levels of Golgi membranes in dendrites and axons similar what we have observed with nicotine exposure to neurons. However, the effects of nicotine required α4β2R expression, while Golgi dispersal regulated by changes in synaptic activity was independent of α4β2R expression. Our findings reveal an activity-regulated Golgi compartment in neurons that appears to function in secretion at local subdomains and contributes to the molecular mechanisms underlying synaptic plasticity.

Disclosures: T.A. Russell: None. **A.P. Govind:** None. **O. Jeyifous:** None. **W.N. Green:** None.

Poster

202. Homeostatic Synaptic Plasticity

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 202.01/B47

Topic: B.07. Synaptic Plasticity

Support: Forschungskredit UZH
SNSF-professorship grant (PP00P3_144816)

Title: Probing the dynamics of presynaptic homeostatic potentiation at the *Drosophila* neuromuscular junction

Authors: *A. G. NAIR, M. MUELLER;
Univ. of Zurich, Zurich, Switzerland

Abstract: Neurotransmission at chemical synapses is under active control of homeostatic mechanisms. These mechanisms contribute to the overall stability of neural network function by conferring synapses with the ability to respond to perturbations. Electrophysiology-based genetic screens at the *Drosophila* neuromuscular junction (NMJ) have identified several genes involved in presynaptic homeostatic potentiation (PHP), a form of homeostatic plasticity that is characterized by the upregulation of presynaptic release upon neurotransmitter receptor perturbation. Though several molecular players have been implicated in PHP, surprisingly little is known about its temporal dynamics and the signalling involved. The ability to experimentally delineate PHP dynamics is partly hampered by currently employed experimental perturbations to induce PHP. While genetic receptor perturbation does not allow studying PHP on short time scales, recordings with the currently used glutamate receptor antagonist Philanthotoxin-433 (PhTx), are confounded by secondary factors, such as irreversibility or activity-dependent receptor blockade. In this study, we therefore sought to develop an experimental protocol that allows assessing the time course of PHP induction and reversal at the *Drosophila* NMJ. To this end, we tested the allosteric glutamate receptor antagonist Gyki-53655 that has been shown to inhibit mammalian AMPA receptors. We found that Gyki-53655 application (10 µM) for ten minutes significantly reduced the amplitude of spontaneous miniature EPSPs (mEPSPs) with respect to controls, indicating a block of *Drosophila* glutamate receptors. Interestingly, the amplitude of action potential (AP)-evoked EPSPs was similar to untreated NMJs. The resulting increase in quantal content suggests that Gyki application induces a homeostatic increase in release within ten minutes. Further experiments revealed that the increase in quantal content is driven by an expansion of the readily-releasable vesicle pool. Gyki washout for 15 minutes resulted in mEPSP amplitudes and EPSP amplitudes that were similar to untreated controls, indicating that both, receptor inhibition and PHP are reversible within 15 minutes. In contrast to PhTX, we find no evidence that Gyki-induced receptor blockade is activity dependent. Together, these two properties of Gyki-dependent receptor inhibition make it well-suited to further explore the dynamics of PHP induction and reversal, as well as the underlying molecular mechanisms with high temporal resolution. Further, pharmacological and genetic perturbations indicate that it recruits presynaptic kinase signaling to upregulate release.

Disclosures: **A.G. Nair:** None. **M. Mueller:** None.

Poster

202. Homeostatic Synaptic Plasticity

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 202.02/B48

Topic: B.07. Synaptic Plasticity

Support: NIH Grant R01NS085164
NSF Grant 1557792
Whitehall Foundation Grant 2014-08-03

Title: The mechanistic transition between the rapid induction and chronic maintenance of homeostatic synaptic plasticity

Authors: *K. M. LEMBKE, C. A. FRANK;
Univ. of Iowa, Iowa City, IA

Abstract: Homeostatic synaptic plasticity (HSP) functions to stabilize neuronal and circuit activities. Failures in HSP are associated with several diseases, including ataxia, epilepsy, and learning and memory defects. For years, HSP was thought to be a slow acting form of neuroplasticity. However, work in the past decade at the *Drosophila melanogaster* neuromuscular junction (NMJ) and other model synapses has shown that homeostatic signaling paradigms can be induced within an acute timescale (minutes) and maintained throughout development. At the NMJ, genetic and pharmacological manipulations can decrease the sensitivity of postsynaptic receptors to single vesicles of neurotransmitter. This decrease in quantal size triggers muscle-to-nerve signaling that drives increased transmitter release. As a result, evoked postsynaptic responses remain steady. Published data have suggested that the two signaling processes (minutes-long induction and days-long maintenance) converge upon the similar presynaptic targets. However, it is unclear how this convergence works. In particular, it is unclear if the long-term maintenance of homeostatic signaling processes at the NMJ is independent of induction - or if it is simply a continuation of induction. For the present study, we exploit those prior findings to address how the short-term induction of HSP gives way to the long-term maintenance of HSP at the NMJ. To do this, we have developed novel methods that combine *Drosophila* genetics with an hours-long pharmacological inhibition of *Drosophila* muscle glutamate receptors. In this way, we are able to re-analyze dozens of previously characterized genetic manipulations that impair homeostatic signaling. The goal of this work is to link previously identified molecules into coherent signaling pathways and to provide temporal resolution of discrete signaling processes that support homeostatic plasticity at the NMJ.

Disclosures: K.M. Lembke: None. **C.A. Frank:** None.

Poster

202. Homeostatic Synaptic Plasticity

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 202.03/B49

Topic: B.07. Synaptic Plasticity

Support: NIH Grant R01N2085164
NSF Grant 1557792
Whitehall Foundation Grant 2014-08-03

Title: Novel links between factors regulating sleep and homeostatic synaptic plasticity

Authors: *N. S. ARMSTRONG, C. A. FRANK;
Univ. of Iowa, Iowa City, IA

Abstract: Sleep is widely conserved among metazoans, but its function is not well understood. One widely-held assumption is that sleep promotes physiological maintenance of synapses, but this has not been thoroughly tested. We hypothesized that factors regulating sleep could be functionally characterized at the *Drosophila melanogaster* neuromuscular junction (NMJ) if they have a conserved synaptic function. Using an RNAi-mediated knockdown of sleep-regulating genes in neurons and muscles, we tested whether candidate genes also have a conserved function in maintaining synaptic stability. After electrophysiologically screening approximately 130 genes, we found less than ten genes that are necessary for maintaining a physiologically normal level of neurotransmission. To evaluate these genes for a potential role in promoting homeostatic synaptic plasticity (HSP), we knocked down each candidate gene in conjunction with an RNAi-based knockdown of a glutamate receptor subunit gene. Of the candidate genes, several were determined to be necessary for neurotransmission, while a small number are required for the maintenance of HSP. These results suggest that a subset of sleep-regulating genes have a conserved function in promoting the physiological maintenance of synapses by preserving baseline neurotransmission and HSP. Data will be presented summarizing our screen and follow-up characteristics.

Disclosures: N.S. Armstrong: None. **C.A. Frank:** None.

Poster

202. Homeostatic Synaptic Plasticity

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 202.04/B50

Topic: B.07. Synaptic Plasticity

Support: NIH Grant NS091546

Title: The sleep gene insomniac is required for rapid ubiquitination at postsynaptic compartments and retrograde homeostatic signaling

Authors: *D. K. DICKMAN[1], K. KIKUMA[1], X. LI[2], P. GOEL[1], S. PERRY[2];
[1]Neurobio., [2]USC, Los Angeles, CA

Abstract: The nervous system confronts challenges during development and experience that can destabilize information processing. To adapt to these perturbations, synapses homeostatically adjust synaptic strength, a process referred to as *homeostatic synaptic plasticity*. At the *Drosophila* neuromuscular junction, inhibition of postsynaptic glutamate receptors activates retrograde signaling that precisely increases presynaptic neurotransmitter release to restore baseline synaptic strength. However, the nature of the underlying postsynaptic induction process remains enigmatic. Here, we designed a forward genetic screen to discover factors in the postsynaptic compartment necessary to generate retrograde homeostatic signaling. This approach identified *insomniac* (*inc*), a putative adaptor for the Cullin-3 (Cul3) ubiquitin ligase complex, which together with Cul3 is essential for normal sleep regulation. Interestingly, we find that Inc and Cul3 rapidly accumulate at postsynaptic compartments following acute receptor inhibition and are required for a local increase in mono-ubiquitination. Finally, we show that Peflin, a Ca^{2+}-regulated Cul3 co-adaptor, is necessary for homeostatic communication, suggesting a relationship between Ca^{2+} signaling and control of Cul3/Inc activity in the postsynaptic compartment. Our study suggests that Cul3/Inc-dependent mono-ubiquitination, compartmentalized at postsynaptic densities, gates retrograde signaling and provides an intriguing molecular link between the control of sleep and homeostatic plasticity at synapses.

Disclosures: **D.K. Dickman:** None. **K. Kikuma:** None. **X. Li:** None. **P. Goel:** None. **S. Perry:** None.

Poster

202. Homeostatic Synaptic Plasticity

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 202.05/B51

Topic: B.07. Synaptic Plasticity

Title: The mechanism of LIMKs/cofilin signaling pathway in NGPF2 regulated homeostatic synaptic plasticity

Authors: *G. HE[1], X. ZHANG[2], Z. ZHOU[1];
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Abstract: Homeostatic synaptic plasticity (HSP) is a form of negative regulation to maintain the network stability in the central nervous system. Although a few molecules and signaling pathway have been proved to regulate the expression of HSP, the molecular mechanism in induction phase is little unknown. In this study, we utilized a sodium channel blocker, tetrodotoxin (TTX) to deprive the cultured primary cortical neuronal activity to explore the HSP. We found that large amount of neurite growth-promoting factor 2 (NGPF2) was secreted into the medium after exposure to TTX for short time. We further explored the underlying mechanism of NGPF2 regulated HSP through a serious of assays, including biochemistry, molecular biology, imaging and electrophysiology. First, we found that NGPF2 can promote the dendritic spine maturation and the strength of synaptic transmission. Second, the TTX-induced NGPF2 expression is regulated by fragile X mental retardation protein (FMRP). Third, we show that NGPF2 is critical for the initiation of HSP, by interacting with anaplastic lymphoma kinase (ALK) thus subsequently activate downstream LIMK/Cofilin pathway, resulted in an increase of Cofilin phosphorylation, which mediate dendritic spine maturation and ultimately regulate HSP. These results indicated that NGPF2 regulates the induction of HSP and reveals LIMKs/Cofilin signaling pathway involved in NGPF2 regulated HSP, which provides a theoretical basis for further study of HSP.
Keyword: Homeostatic Synaptic Plasticity, Synapse, Neurite Growth-promoting Factor 2, LIM Kinase, Cofilin

Disclosures: **G. He:** None. **X. Zhang:** None. **Z. Zhou:** None.

Poster

202. Homeostatic Synaptic Plasticity

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Program #/Poster #: 202.06/B52

Topic: B.07. Synaptic Plasticity

Support: Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (19K17117)
Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (15K19742)

Title: Social isolation affects inhibitory neural circuits of prefrontal cortex during development

Authors: *K. OKAMURA[1], S. KIMOTO[1], H. YOSHINO[1], Y. NISHIHATA[1], Y. YAMAGUCHI[1], K. YAMAMURO[1], M. IKEHARA[1], M. MAKINODAN[1], Y. OGAWA[2], Y. SAITO[2], T. KISHIMOTO[1];
[1]Dept. of Psychiatry, [2]Dept. of Physiol. 1, Nara Med. Univ., Kashiara, Nara, Japan

Abstract: Study objectives: Social experiences are essential for the development of mammal brain. Impairments in cognition and sociability, which are largely influenced by social milieu, are considered core elements across psychiatric disorders such as schizophrenia and autistic spectrum disorder. We previously reported mice isolated only for 2 weeks immediately after weaning developed deficits in sociability and working memory, and hypomyelination in the deep layers of the prefrontal cortex (PFC). These alterations were also characterized by reduced excitatory synaptic inputs to a subtype of pyramidal cells in layer 5. However, progression mechanisms in the immature PFC in isolation-rearing remain unclear. Therefore, we sought to perform electrophysiological and molecular analysis to determine the developmental effects of isolation-rearing on PFC. Methods and material: Male C57/BL6 mice and one commercially available transgenic mouse (GAD67-GFP mouse) were used in the present study. After weaning at postnatal day 21 (P21), 4 male littermates were randomly divided into 1 isolated mouse and 3 group-reared mice for 2 weeks. Brain slices, including PFC region, were prepared for the electrophysiological analysis at the three time points from P21, P35 and P65, respectively. Then, the effect on membrane properties on layer 5 pyramidal cells and parvalbumin (PV) positive cells were assessed by whole-cell patch-clamp recording. Finally, mRNA and protein expression of PV were compared between socially-isolated mice and group-reared mice. Results: In socially-isolated mice, frequency of the excitatory synaptic inputs onto a subtype of pyramidal cells in layer 5 was reduced at P65, but not at P35. In contrast, frequency of the excitatory synaptic inputs onto PV positive cells was higher than group-reared mice at P35. Consistent with electrophysiological evaluation in PV positive cells, expression levels of PV were progressively decreased after the isolation-rearing.

Conclusions: The present study suggests dysfunction of PV neurons might precede that of pyramidal neurons in socially-isolated mice, providing a greater insight into understanding the mechanisms of prefrontal function driven by social experience.

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Poster

202. Homeostatic Synaptic Plasticity

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 202.07/B53

Topic: B.07. Synaptic Plasticity

Support: European Research Council Grant 724866
Legacy Heritage Biomedical Program of the Israel Science Foundation Grant 1849/17
Israel Science Foundation Grant 1663/18

Title: IGF1 receptor is necessary for firing rate homeostatic recovery in response to inactivity

Authors: *M. KATSENELSON[1,2], I. SHAPIRA[1], S. AÏD[3], I. SLUTSKY[2,1];
[1]Physiol. & Pharmacol., [2]Sagol Sch. of Neurosci., Tel Aviv Univ., Tel-Aviv, Israel; [3]Res. Ctr. UMR 938, INSERM and Sorbonne Univ., Paris, France

Abstract: Neuronal circuits achieve an ongoing balance between stability and flexibility under constantly changing environment. Prolonged changes in neuronal activity trigger a wide spectrum of homeostatic mechanisms, stabilizing mean firing rate (MFR) of neuronal populations around a set-point value. While numerous homeostatic effectors that drive compensatory changes have been uncovered, the master regulator orchestrating all the homeostatic responses remains unknown. Insulin-like growth factor 1 receptor (IGF1R) is an evolutionary-conserved regulator of diverse processes such as proteostasis, and energy and calcium homeostasis. This has brought us to hypothesize that IGF1R might play a central role in firing homeostasis of central neural circuits. To investigate the role of IGF1Rs in MFR homeostasis, we combined long-term recordings of spikes using micro-electrode arrays, together with patch-clamp measurements and imaging of synaptic signaling under highly-controlled environment of cultured hippocampal networks. When left unperturbed, MFRs are maintained around a set-point value for hours and days of recording. As previously established, chronic inhibition by baclofen, the $GABA_B$ receptor agonist, triggered a drop in the MFR that was renormalized within 2 days in the presence of the perturbation. Surprisingly, when IGF1R was inhibited either genetically or pharmacologically, the inhibition by baclofen was persistent and

MFR did not renormalize during 2 days of perturbation. Moreover, no compensation was observed at the level of excitatory quantal synaptic transmission or intrinsic excitability of hippocampal neurons. Currently, we are investigating the cellular and molecular mechanisms underlying regulation of homeostatic feedback responses by IGF1R. Taken together, our results highlight IGF-1R as a key regulator of homeostatic response, orchestrating multiple adaptive mechanisms that are essential for recovery of MFR in response to chronic inactivity.

Disclosures: M. Katsenelson: None. **I. Shapira:** None. **S. Aïd:** None. **I. Slutsky:** None.

Poster

202. Homeostatic Synaptic Plasticity

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Topic: B.07. Synaptic Plasticity

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Title: Role of IL-33/ST2 signaling in homeostatic synaptic plasticity in the hippocampus

Authors: *Y. WANG[1,2,3], K.-W. HUNG[1,2,3], C.-Y. CHUANG[1,2,3], W.-Y. FU[1,2,3], A. K. Y. FU[1,2,3], N. Y. IP[1,2,3];
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Abstract: Synaptic plasticity, a cellular mechanism characterized by activity-dependent changes in synaptic strength, underlies learning and memory. Homeostatic synaptic plasticity, also known as “synaptic scaling”, is the ability of neuronal synapses to compensate in response to prolonged changes in neuronal activity. Dysfunction of synaptic scaling might contribute to cognitive impairment in neurodegenerative diseases such as Alzheimer’s disease. Emerging evidence suggests that several immune molecules expressed in the central nervous system play important roles in synapse development and plasticity. However, it is unclear how these immune molecules regulate synaptic functions. Here, we found that interleukin (IL)-33, an inflammatory cytokine, regulates synaptic scaling in the mouse hippocampus. Specifically, inhibition of IL-33 signaling attenuated activity blockade-induced synaptic upscaling in cultured hippocampal neurons, as

indicated by the abolishment of TTX-induced increase in excitatory synaptic transmission in neurons treated with soluble ST2 (sST2), a decoy receptor for IL-33. Moreover, IL-33 administration enhanced excitatory synaptic transmission, while knockdown of IL-33 receptor complex, ST2, or IL-1RAcP, abolished this enhancement, indicating that IL-33 mediates synaptic upscaling via signaling dependent on ST2/IL-1RAcP in hippocampal neurons. These findings demonstrate that IL-33/ST2 signaling is essential for synaptic homeostasis in the hippocampus.

Disclosures: **Y. Wang:** None. **K. Hung:** None. **C. Chuang:** None. **W. Fu:** None. **A.K.Y. Fu:** None. **N.Y. Ip:** None.

Poster

202. Homeostatic Synaptic Plasticity

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Program #/Poster #: 202.09/B55

Topic: B.07. Synaptic Plasticity

Support: DFG (SFB 1089, SPP 1757, SCHO 820/4-1, SCHO 820/6-1, DI853/3-2, DI853/7-1)
BONFOR

Title: Phosphorylation in presynaptic plasticity - SRPK2 as a relevant kinase

Authors: ***A. MAYER**[1], J. BETZIN[1], J. MÜLLER[1], D. DIETRICH[2], S. SCHOCH[1];
[1]Inst. of Neuropathology, Dept. of Epileptology, [2]Dept. of Neurosurg., Univ. of Bonn Med. Ctr., Bonn, Germany

Abstract: Synaptic plasticity is the ability of synapses to adapt to activity- and experience-dependent changes by altering their strength and the efficacy of synaptic transmission. This synaptic strength can change short term as well as long term and thereby contributes to adaptions to sensory inputs as well as learning and memory. The phosphorylation states of synaptic proteins, especially those regulating vesicle exocytosis are presumably playing an important role in synaptic plasticity and whereas phosphorylation mechanisms on the postsynaptic site are well described, the role in presynaptic plasticity is still rather unknown.
One kinase, which has been identified to trigger phosporylation of several presynaptic active zone proteins, is the SR protein kinase 2 (SRPK2). Therefore we tested whether the overexpression (OE) or knock-down (KD) of SRPK2, which was induced by transduction with rAAV particles, has an effect on homeostatic plasticity. Using the genetically encoded glutamate sensor iGluSnFR we investigated whether SRPK2 is required for homeostatic up-scaling of synaptic release after silencing hippocampal cultures of primary neurons with TTX (48 hrs). Untreated cultures showed a strong increase in transmitter output (by 60 ± 20%), which was

abolished upon OE of SPRK2 (increase is completely eradicated, decrease in transmitter output by 10%). Interestingly, also SRPK2 knock-down decreased homeostatic up-scaling although to a lower extend. This data suggests that regulation of SRPK2 presynpatic activity or levels is important for presynaptic homeostatic plasticity.

Disclosures: **A. Mayer:** None. **J. Betzin:** None. **J. Müller:** None. **D. Dietrich:** None. **S. Schoch:** None.

Poster

202. Homeostatic Synaptic Plasticity

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Topic: B.07. Synaptic Plasticity

Support: Simons Foundation Award #: 345485 (GGT)
R37NS092635 (GGT)

Title: Phosphorylation state of Shank3 is a critical regulator homeostatic scaling

Authors: *V. TATAVARTY[1], C.-H. WU[2], F. F. WAGNER[4], J. R. COTTRELL[5], G. TURRIGIANO[3];
[2]Biol., [3]Dept of Biol., [1]Brandeis Univ., Waltham, MA; [4]Stanley Ctr. for Psychiatric Res., Broad Inst., Cambridge, MA; [5]Stanley Ctr. for Psychiatric Res., Broad Inst. of MIT and Harvard, Cambridge, MA

Abstract: Homeostatic plasticity mechanisms such as synaptic scaling prevent runaway excitation or silencing of neural networks (Turrigiano 2017). Deficits in homeostatic plasticity may play a critical role in the pathogenesis of Autism Spectrum Disorders (ASD)(Valakh and Nelson 2015). We investigated an ASD linked gene Shank3 for its role in regulating homeostatic synaptic scaling. Shank3 knockdown via short hairpin expression in visual cortical neurons blocks synaptic scaling. Interestingly, scaling deficits caused by reduction in Shank3 levels in cortical neurons can be rescued by lithium treatment. Using a proteomic approach, we have identified phosphorylation sites on Shank3 that are bidirectionally regulated during the induction of synaptic scaling. Scaling up leads to a decrease in phosphorylation of Shank3 while scaling down is marked by an increase in Shank3 phosphorylation. We go on to demonstrate that these Shank3 phosphorylation sites are sensitive to lithium treatment as well as GSK3 inhibition. Electrophysiological recordings in cultured cortical neurons expressing non-phosphorylatable Shank3 mutants lead to a block of scaling down. Conversely phosphor-mimetic Shank3 mutations prevent the induction of scaling up. We are currently investigating how Shank3 phosphorylation states influence its protein-protein interactions as well as the stability of Shank3.

These results suggest that regulation of Shank3 phosphorylation may play an important role in ASD and provide a target for directed pharmacological intervention.

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Poster

202. Homeostatic Synaptic Plasticity

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Topic: B.07. Synaptic Plasticity

Support: NIH Grant NS083402
NSF training Grant 1735252

Title: Regulation of Kv7 and Nav channels at the axon initial segment of hippocampal neurons during homeostatic scaling of intrinsic excitability

Authors: *B. BACULIS[1], A. ZHU[2], D. CHAO[2], Z. HUANG[3], K. LEE[3], H. CHUNG[3]; [1]Neurosci., [2]Mol. and Cell. Biol., [3]Mol. Integrative Physiol., Univ. of Illinois Urbana-Champaign, Urbana, IL

Abstract: Homeostatic plasticity maintains neuronal activity within a physiological range. Homeostatic scaling of intrinsic excitability is thought to be involved in a variety of neurological diseases including epilepsy. Animal models of epilepsy have shown changes in voltage-gated ion channels localized at the axon initial segment (AIS) and have been linked to epileptogenesis. Our lab has previously shown that upon prolonged activity blockade using TTX or a reduction in calcium influx through NMDAR using APV leads to homeostatic scaling of intrinsic excitability in dissociated hippocampal culture. In addition, we see a reduction in mRNA, protein expression, and current of voltage-gated potassium (Kv7) channels (Lee et al., 2015). Kv7 channels are slow activating, non-inactivating channels that are critical for preventing repetitive firing of action potentials. Kv7 and voltage-gated sodium channel (Nav1) bind to the AIS scaffolding protein ankyrin-G and are required for the normal function of the AIS. Casein Kinase 2 (CK2) phosphorylation has been found to be regulated in-directly by activity and calcium via Ca2+/Calmodulin-dependent protein kinase II. CK2 has also been found to be enriched at the AIS and has been found to alter Kv7 and Nav1 binding to ankyrin-G. However, It is unknown if a reduction in CK2 phosphorylation of Kv7 and Nav1 at the AIS is responsible for homeostatic scaling of intrinsic excitability after activity blockade. My preliminary results indicate activity-dependent changes in the expression of ankyrin-G and Nav1 upon 48-hour activity blockade by TTX and APV in dissociated hippocampal culture. There is a decrease in the mean intensity of ankyrin-G along with an increase in total length and a shift towards the soma. PanNav shows a

similar change. Based on these results we are currently testing the hypothesis that CK2 is involved in scaling of intrinsic excitability. We will overexpress wild type CK2, kinase in-active CK2 (K68M), and empty vector control in dissociated culture to test if a reduction in CK2 phosphorylation is sufficient to cause scaling of intrinsic excitability and the changes in ankyrin-G, Nav1, and Kv7 localization and expression at the AIS as seen in activity blockade.

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Poster

202. Homeostatic Synaptic Plasticity

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Program #/Poster #: 202.12/B58

Topic: B.07. Synaptic Plasticity

Support: NIH 5R90DA033462

Title: Neuronal and synaptic parameter degeneracy in central pattern generator activity

Authors: *K. TIAN, A. A. PRINZ;
Dept. of Biol., Emory Univ., Atlanta, GA

Abstract: Degeneracy in neural systems refers to the phenomenon that the same neural activity or behavior can be achieved through multiple combinations of the underlying parameters. For example, multiple combinations of intrinsic and synaptic current densities can produce the same pyloric rhythm in the crab *Cancer borealis* pyloric pattern generating circuit [1-2]. This phenomenon has been observed widely in vertebrate and invertebrate systems and appears to contribute to robustness against injuries or perturbations. However, there is still a lack of a theoretical framework to define and quantify degeneracy.
We propose a novel measure of degeneracy by combining a method called filtration from algebraic topology with Pearson's r and apply it to understand the role of degeneracy in maintaining the pyloric rhythm after deafferentation, a perturbation that blocks all descending neuromodulators and leads to an initial loss of rhythmic activity, followed by rhythm recovery several days later. We first employ an ensemble modeling approach to find multiple combinations of intrinsic and synaptic parameters that can produce pyloric rhythms observed at two stages of the rhythm recovery behavior, which are control and four days after deafferentation [3]. Then we quantify degeneracy of the parameter sets at each stage and find that compared to control, parameter sets at four days after deafferentation show increased degeneracy between intrinsic parameters and reduced degeneracy between intrinsic and synaptic parameters, indicating cell-autonomous mechanisms of maintaining the pyloric rhythm after deafferentation.
Acknowledgments

This work is supported by NIH Training Grant 5R90DA033462, and the simulation was performed on the Neuroscience Gateway Portal [4].
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2. Ransdell *et al.* (2013) Journal of Neurosci 33(24): 9950-9956
3. Prinz (2010) Phil. Trans. R. Soc. B 365:2397-2405.
4. S Sivagnanam, A Majumdar, K Yoshimoto, V Astakhov, A Bandrowski, M. E. Martone, and N. T. Carnevale. Introducing the Neuroscience Gateway, IWSG, volume 993 of CEUR Workshop Proceedings, CEUR-WS.org, 2013

Disclosures: K. Tian: None. **A.A. Prinz:** None.

Poster

202. Homeostatic Synaptic Plasticity

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Topic: B.07. Synaptic Plasticity

Support: the National Nature Sciences Foundation of China (NSFC 31871041)
Zhejiang Provincial Natural Science Foundation of China (LY17C090007)

Title: Visual experience facilitates excitatory synaptic scaling via Rab5C-mediated enhancement of AMPA receptor internalization

Authors: *W. SHEN, L. ZHENG, Z. GUO, X. QI, Y. LIAO, Y. SONG;
Hangzhou Normal Univ., Hangzhou, China

Abstract: Neurons counteract over-excitation or inhibition by regulating synaptic intensity to maintain homeostatic plasticity through a negative feedback mechanism. Visual experience is thought to play an important role in synaptic scaling, neural development and circuit plasticity, however, the effect of elongated or shortened day time exposure to synaptic function and underlying mechanism remain unclear. Using Xenopus laevis tadpoles as model animals, we studied the changes of excitatory synaptic transmission induced by increasing (20h light:4h dark; 20LE) or decreasing (4h light:20h dark; 4LE) visual input through changing the duration of light exposure. We find that amplitudes of mEPSCs are gradually decreased from stage 42 to 49 in the developing tectal neurons, whereas 20LE treated tadpoles showing facilitated decrease of amplitudes. Paired pulse ratio is not altered among 12LE, 20LE and 4LE tadpoles, and visually-evoked excitatory synaptic currents are decreased in 20LE tectal neurons, suggesting the main effect of postsynaptic receptors recycling on synaptic scaling. Using RNA sequencing with RT-PCR technique, we identify Rab family members that are increased in tadpole brain over 2 days of 20LE. Concordantly, GluA1 and GluA2 expression are significantly decreased while Rab5c

expression is greatly increased in 20LE tadpoles by Western blotting. The dynamic control of scaling down in excitatory synaptic transmission in 20 LE tadpoles is reversed by knockdown of Rab5c with a morpholino against Rab5c or re-expose tadpoles to 12LE for 2 days. Long day exposure-induced increase of visual avoidance index is maintained in 20LE for 7 days, however, short day exposure only results in acute increase of avoidance index, suggesting that scaling down in excitatory synaptic transmission through rab5c-dependent AMPA receptor trafficking is required to promote neural circuit maturation. We demonstrate that excitatory synaptic scaling down in individual neurons are sculpted by visual activity and mediated by Rab5c-dependent AMPA receptor recycling.

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Poster

202. Homeostatic Synaptic Plasticity

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Program #/Poster #: 202.14/B60

Topic: B.07. Synaptic Plasticity

Support: 5R01EY025613-02

Title: Sleep drives downward firing rate homeostasis in V1 neurons

Authors: *A. TORRADO PACHECO, J. BOTTORFF, G. TURRIGIANO;
Dept of Biol., Brandeis Univ., Waltham, MA

Abstract: Homeostatic plasticity mechanisms act to keep the activity of individual neurons within a target set-point, a process known as firing rate homeostasis (FRH). Earlier work from our lab using chronic *in vivo* recordings first demonstrated upward FRH neurons in primary visual cortex (V1) of freely behaving rats following monocular deprivation (MD). We found that this upward recovery only happens while the animal is awake. This raises important questions about the extent to which firing rates are homeostatically regulated in the brain, and the role that sleep and wake states play in this process. To address this, we used *in vivo* electrophysiology in freely behaving rats and performed eye re-opening (ER) after 5 days of MD. This causes activity in V1 to increase above its pre-MD baseline in the first 24-36 hours, but FRs then recover to their pre-MD baseline levels within an additional 3 days. This is the first demonstration that downward FRH happens *in vivo*, and that FRH is bi-directional. We use pharmacology to show that the different phases of FR change, i.e. the overshoot immediately after ER and subsequent downward recovery, are driven by different plasticity mechanisms: the overshoot phase is NMDA receptor-dependent, while the recovery phase is not. These data suggest that the recovery phase of FRH is driven by homeostatic plasticity mechanisms, which are independent of NMDA

receptor activation. We scored animals' behavior across the recording to explore the regulation of FRH by sleep and wake. In contrast to our findings on upward FRH, we show that the downward homeostatic recovery of activity in the days following ER happens during periods of sleep. Sleep-dense epochs (>70% sleep) are associated with a significant decrease in FRs, while wake-dense periods show no change. During periods of extended sleep (>30 min) firing rates decreased in V1 neurons contralateral to the re-opened eye, but not in ipsilateral control neurons. Periods of extended wake had no effect. We similarly analyzed the decrease in FR caused by MD. In this case, we did not find a dependence on behavioral state; instead, FR decreases were associated with time spent in light (vs darkness), suggesting an activity-dependent process. These data reveal a complex regulation of FRs *in vivo*, where homoestatic changes in different directions are gated by different behavioral states. This is an important advance in our understanding of how plasticity mechanisms are implemented in the brain, and suggests that sleep and wake states may gate up- and down-scaling by differentially affecting specific molecular players, or via the effects of neuromodulation.

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Poster

202. Homeostatic Synaptic Plasticity

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Topic: B.07. Synaptic Plasticity

Support: NIH grant R21NS096483
NIH grant R01NS107424
Neurology department develoment fund

Title: Aldehyde dehydrogenase involves in cortical neuron up-state potentiation of spontaneous excitatory synaptic currents in IGE models

Authors: *C. ZHOU[1], L. DING[2], C. HANNA[2], M. J. GALLAGHER[4], R. L. MACDONALD[3]; [2]Neurol., [3]Professor Dept Neurol, [1]Vanderbilt Univ. Med. Ctr., Nashville, TN; [4]Neurol., Vanderbilt Univ. Sch. of Med., Nashville, TN

Abstract: Cortical neurons undergo up/down-state alternation *in-vivo* during slow-wave sleep while brain exhibits 0.5 Hz EEG oscillatory waveforms (slow-wave oscillations, SWOs). During up-states, neurons depolarize and fire action potentials(APs). Depending on precedent activity, neurons can potentiate or depress synapse strength by homeostatic plasticity mechanisms (Turrigiano 2008; Pozo and Goda 2010; Bartram et al., 2017). Moreover, homeostatic potentiation of synaptic currents depends on retinoid acid synthesis which can be blocked with inhibitors of aldehyde dehydrogenase(ALDH) such as N,N-diethylaminobenz- aldehyde(DEAB)

(Aoto et al., 2008; Chen et al., 2014).
Previously we injected 0.5 Hz cosine currents into cortical neurons (in brain slices) to simulate SWOs *in-vivo*, finding that spontaneous(s) excitatory synaptic currents(sEPSCs) (not inhibitory currents) could be enhanced, resulting in unbalanced/enhanced sEPSCs in our idiopathic generalized epilepsy (IGE) models. Here we used physiology-like up/down-state alternation to examine whether sEPSCs in neurons could still be potentiated or not in *ex-vivo* brain slices. This makes it feasible for us to test effect of DEAB to suppress sEPSC potentiation for a potential therapeutic treatment of seizures in IGE models.
We used whole-cell patch-clamp recordings of sEPSCs in cortical neurons within *ex-vivo* brain slices from knock-in (KI) IGE mouse models with GABR$\alpha 1^{A322D}$ or GABR$\gamma 2^{Q390X}$ mutation(heterozygous(het)). Neuron up/down-state alternation was induced by using one modified ACSF containing (mM) 3.5 or 5 KCl, 1 Ca^{2+}, 1 Mg^{2+}, together with 3.5 µM carbachol and fast flow perfusion (6~7ml/min, small volume chamber, at 32~33°C). The induced up-states in neurons could maintain more than 5~10 seconds with neuron firing APs. Then neurons hyperpolarized until next up-state cycle started. Consistent with our previous findings, induction of up-states (7~10 min) in pyramidal neurons could potentiate sEPSCs(from 17.40±2.46 pA to 27.84±3.52, n=6, paired t-test p=0.004) from het KI or WT littermate mice. In contrast, with 30~40 µM DEAB included in ACSF (at least for 2~3 hours), up-state induction could not potentiate sEPSCs (from 22.41±1.72 pA to 20.53±1.49 pA, n=4, p>0.05) in pyramidal neurons from het KI or WT mice. In addition, inclusion of DEAB in ACSF did not affect neuron baseline spontaneous sEPSCs and inhibitory currents. Together these results indicated that ALDH contributed to sEPSC potentiation by up-state induction in neurons and inhibition of ALDH by DEAB could suppress escaped sEPSCs during SWOs, which may offer one potential therapeutic treatment of IGE epileptic activity.

Disclosures: **C. Zhou:** None. **L. Ding:** None. **C. Hanna:** None. **M.J. Gallagher:** None. **R.L. Macdonald:** None.

Poster

202. Homeostatic Synaptic Plasticity

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Program #/Poster #: 202.16/B62

Topic: B.07. Synaptic Plasticity

Support: NRF-2017M3C7A1029611
18-BR-03-02

Title: Altered synaptic scaling of PV^+ interneurons underlying sensorimotor gating deficit

Authors: ***J. SHIN**[1,2], S. KIM[1,2], Y. KIM[1], H. LEE[2], Y. HARUNA[3], H.-S. SHIN[2], J. PARK[2], S. KIM[1];

[1]Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Ctr. for Cognition and Sociality, Inst. for Basic Sci., Daejeon, Korea, Republic of; [3]Tokyo Med. and Dent. Univ., Tokyo, Japan

Abstract: Sensorimotor gating is disrupted in psychiatric disorders, particularly schizophrenia. Several pathophysiological evidences are consistent with dysfunction in sub-population of inhibitory interneurons including parvalbumin-positive interneurons (PV^+IN), leading to impairments of inhibitory gating on sensory processing at the neuronal network. However, PV^+IN mechanisms underlying reduced sensorimotor gating at a cellular and micro-circuit level remain unclear. We investigated two mouse models of schizophrenia-relevant psychiatric disorders: chronic NMDA receptor hypofunction model with repeated mk-801 (MK) administration and *pvalb*, an impacted hallmark of schizophrenia, knockdown modeling (PV-KD). A pre-pulse inhibition assay confirmed a sensorimotor gating deficit in two mouse models. In local field potential recording at prelimbic (Pr) with optogenetic modulation of PV^+IN, suppression of PV^+IN induced gamma-band oscillation (GBO) abnormalities. This phenomenon of the reduced power of GBO was present in two mouse models and also was rescued by excitation of PV^+IN. *Ex vivo* electrophysiological recording revealed synaptic downscaling of AMPA receptors in PV^+IN but not in the other neurons, leading to excitation/inhibition (E/I) imbalance. Molecular assays confirmed that reduction of adenylyl cyclase 5 (AC5) mediated PKA signaling results in aberrant synaptic scaling specifically in PV^+IN. Pr targeting infusion of AC5 specific activator improved dysfunction of sensorimotor gating, associated with recovery of GBO power and synaptic upscaling in PV^+IN. These observations demonstrate that AC5 activity-dependent PKA signaling underlies modulation of synaptic scaling in PV^+IN, significant in the understanding of the pathophysiology of sensorimotor gating deficit.

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Poster

202. Homeostatic Synaptic Plasticity

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Topic: B.07. Synaptic Plasticity

Support: CIHR Grant

Title: Synaptopodin's role in homeostatic upscaling

Authors: *J. BOATENG, M. CHAN, P. K. Y. CHANG, J. POPIC, R. MCKINNEY; McGill, Montreal, QC, Canada

Abstract: Mechanisms that underlie learning and memory can destabilize neural networks when left uncontrolled. Homeostatic scaling is a process that maintains the stability of neural networks through the trafficking of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR) at excitatory synapses. Dendritic spines possess the relevant cellular machinery for AMPAR trafficking and within a subset of larger dendritic spines is synaptopodin, an actin-associated protein. While synaptopodin has been documented to promote Hebbian plasticity and is involved in AMPAR trafficking, its function within the context of homeostatic synaptic scaling is not understood. Here, we set out to investigate the role of synaptopodin in homeostatic scaling. Scaling was induced by treating wildtype (WT) and synaptopodin knockout (SPKO) organotypic hippocampal cultures with tetrodotoxin (TTX) for 3-4 days. Whole cell electrophysiology was used to measure AMPA mediated miniature excitatory postsynaptic currents (mESPC). While WT CA1 pyramidal neurons scaled up the amplitude of mESPC, SPKO neurons were unable to undergo upscaling. Release of tumor necrosis factor alpha (TNFα), a factor necessary for upscaling, was observed in WT neurons but not SPKO neurons during chronic inactivity. The addition of exogenous TNFα did not restore scaling in SPKO neurons despite the expression and activation of the functional receptor. Both WT and SPKO neurons were able to induce translocation of nuclear factor kappa B (NFkB) to the nucleus and elevation of nuclear CAMKIV after addition of exogenous TNFα. This evidence suggests that the machinery necessary for transcription of new AMPARs is intact in SPKO neurons. However, preliminary data showing low levels of surface AMPARs in SPKO neurons after TTX treatment indicates an impairment in AMPAR trafficking. Considering that synaptopodin is scaffolding protein, its loss can impair the localization of PKA and CAMKII, two kinases that mediate the trafficking of AMPARs, leading to the inability of SPKO neurons to induce scaling.The findings from this study show that synaptopodin is necessary for synaptic scaling. It contributes to the evolving literature on homeostatic plasticity and enhance our knowledge of the potential mechanisms involved.

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Poster

202. Homeostatic Synaptic Plasticity

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Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 202.18/B64

Topic: B.07. Synaptic Plasticity

Support: CIHR
NSERC

Title: Astrocyte production of TNF drives homeostatic synaptic plasticity

Authors: *D. STELLWAGEN[1], R. HEIR[2], H. ALTIMIMI[1], M. C. FRANQUIN[3], P. KOMAL[4], J. CHAMBON[1];
[1]McGill Univ., Montreal, QC, Canada; [2]McGill Univ. Ctr. For Res. In Neurosci., Montreal, QC, Canada; [3]Ctr. For Res. In Neurosci., McGill Univ., Montreal, QC, Canada; [4]Dept. of Biol., BITS-Pilani Hyderabad, Telangana, India

Abstract: For neural circuits to function well, overall activity levels must be kept within an optimal range by homeostatic synaptic plasticity (HSP) mechanisms. The process of homeostatic strengthening of excitatory synapses in response to chronic activity deprivation is mediated by the glial release of tumour necrosis factor alpha (TNF), which modulates both AMPA and GABA synaptic receptor trafficking. While glia play a critical role in some forms of HSP, the glial source—whether from astrocytes or microglia—has been unresolved. Here we show that astrocytes supply TNF during HSP in dissociated cultures, and that this occurs at least in part through regulation of TNF mRNA levels. We show that 48 hour activity deprivation of cultures with tetrodotoxin (TTX) results in both an increase in TNF mRNA levels as well as an increase in surface GluA1 levels. Depletion of microglia from these cultures does not prevent TNF release in response to TTX, nor does it prevent the increase in surface GluA1, suggesting that astrocytes are producing TNF in this context. In addition, we use organotypic hippocampal slice cultures to investigate glial release of TNF in a situation more closely representing *in vivo* conditions. We find that in slice cultures where TNF is genetically deleted from microglia, mEPSC amplitude still increases in response to activity deprivation, suggesting that microglial TNF is not necessary for this effect. In contrast, astrocytic deletion of TNF does prevent the increase in mESPC amplitude. We therefore show that in multiple culture systems, astrocytes are capable of supplying the TNF that results in HSP. Astrocytes can respond to extracellular glutamate through mGluRs to control TNF levels through NFkB signaling. This suggests that during activity deprivation, the absence of synaptic glutamate activates NFkB, resulting in TNF expression and subsequent synaptic strengthening. Finally, we performed a screen for additional cytokines involved in synaptic scaling. Using either 48h TTX treatment in hippocampal slice cultures to decrease activity, or gabazine (GBZ) to increase it, we identify >30 cytokines that are modulated in at least one of the conditions. Future work will define potential new roles for cytokines in activity-dependent synaptic modulation.

Disclosures: D. Stellwagen: None. **R. Heir:** None. **H. Altimimi:** None. **M.C. Franquin:** None. **P. Komal:** None. **J. Chambon:** None.

Poster

202. Homeostatic Synaptic Plasticity

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 202.19/B65

Topic: B.07. Synaptic Plasticity

Title: Investigating the roles of homeostatic synaptic scaling in cortex-dependent associative learning

Authors: *C.-H. WU[1], R. A. RAMOS[1], D. B. KATZ[2], G. TURRIGIANO[1];
[1]Dept. of Biol., [2]Dept. of Psychology, Brandeis Univ., Waltham, MA

Abstract: Hebbian plasticity mechanisms such as long-term potentiation (LTP) are widely considered to underlie learning and memory formation. While important, computational models predict that if left uncontrolled, LTP initiates a positive feedback process that pervasively increases synaptic strengths, and could result in a loss of the synapse specificity that governs proper learning. Synaptic scaling, a form of homeostatic plasticity, has been theorized to constrain this run-away LTP by globally adjusting synaptic strengths in a post-synaptic manner. Compelling as this model is, it remains elusive whether synaptic scaling partners with LTP *in vivo* to ensure the correct encoding of memories. Furthermore, the impact of disrupted synaptic scaling on memory fidelity has not been tested. Here we used an associative-learning paradigm, conditioned taste aversion (CTA) to test these ideas. We hypothesize that perturbation of synaptic scaling in gustatory cortex (GC), the brain region involved in both acquisition and maintenance of CTA, will degrade the stimulus-specificity of CTA. We first characterized the general aversion that arises immediately following CTA training in Long-Evans rats. We demonstrate that after conditioning, young rats form transient aversions to both conditioned and unconditioned tastants. We show that this general aversion is rapidly "sculpted away" to become more specific to the conditioned tastant, and the duration of the transient general aversion correlates with the strength of conditioning. Next, we used an immunofluorescence double-labeling approach to investigate the activation of neuronal ensembles in GC during this process. Our preliminary result suggests that during the period of generalization, neurons activated during conditioning are more likely to be reactivated when rats experience the unconditioned tastant. Lastly, to elucidate the roles of synaptic scaling in the phases of CTA learning, we sought to perturb homeostatic synaptic scaling *in vivo* using viral vectors to introduce either the C-terminus fragment of GluA2 or a mutant GluA2, both known to block synaptic scaling *in vitro*. We found that blocking synaptic scaling in GC enhanced CTA-induced general aversion without impairing the acquisition of CTA. Together, our work establishes that synaptic scaling is important for transition between general and stimulus-specific associative learning.

Disclosures: C. Wu: None. **G. Turrigiano:** None. **R.A. Ramos:** None. **D.B. Katz:** None.

Poster

202. Homeostatic Synaptic Plasticity

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 202.20/B66

Topic: B.07. Synaptic Plasticity

Support: NIH NINDS-R01NS065992

Title: Homeostatic recovery of embryonic spinal activity initiated by compensatory changes in resting membrane potential

Authors: *C. E. GONZALEZ-ISLAS[1,2], P. A. WENNER[1];
[1]Physiol., Emory Univ. Sch. of Med., Atlanta, GA; [2]Ciencias Biologicas, Univ. Autonoma de Tlaxcala, Tlaxcala, Mexico

Abstract: When spiking activity in a neuronal circuit is modified by external challenges, mechanisms are engaged that homeostatically restore activity to its original level. In order to identify the most relevant homeostatic mechanisms, we have taken advantage of a system in which we can monitor a homeostatic recovery of activity in the living circuit, the chick embryo spinal cord. We have previously shown that 2-day blockade of either excitatory GABAergic or glutamatergic transmission in the living embryo transiently blocks the movements generated by spontaneous network activity in the spinal cord. However, after 2 hours of persistent receptor blockade, embryonic movements began to recover and by 12 hours complete homeostatic recovery of movements was observed. Compensatory changes in voltage-gated conductances were previously reported after 12 hours of blockade, although synaptic scaling was not observed at this point. It was unclear whether these changes existed at earlier times of receptor blockade when movements begin to recover. Furthermore, no compensatory changes in voltage-gated conductances or scaling have been observed at any time following glutamatergic blockade, where embryonic movements were transiently blocked but then recovered similarly to that after GABAergic blockade. In this study, we are reporting a novel mechanism for homeostatic recovery that takes place in these first hours of neurotransmitter receptor blockade. Following 2-6 hours of GABAergic or glutamatergic blockade we found a clear reduction in action potential threshold that was mediated by a depolarization of the resting membrane potential in both motoneurons and interneurons. These changes were only observed in the presence of the transmitter antagonist. Therefore, it appears that fast changes in resting membrane potential represent a key fast homeostatic mechanism for the maintenance of network activity in the living embryonic nervous system.

Disclosures: C.E. Gonzalez-Islas: None. **P.A. Wenner:** None.

Poster

202. Homeostatic Synaptic Plasticity

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 202.21/B67

Topic: B.07. Synaptic Plasticity

Support: NIH, NINDS Grant R01NS065992

Title: Relationship between quantal amplitude and evoked response during synaptic scaling in embryonic spinal motoneurons

Authors: *D. PEKALA, P. WENNER;
Emory Univ. Sch. of Med., Atlanta, GA

Abstract: CNS neurons possess the ability to adjust the strength of their synapses in response to a perturbation in order to stabilize neuronal signaling through a form of homeostatic plasticity known as synaptic scaling. In chick embryonic spinal motoneurons, activity deprivation causes a compensatory increase in the amplitude of excitatory miniature postsynaptic currents (mEPSCs), also known as quantal amplitude. It has been assumed that increases in quantal amplitude observed during synaptic scaling may translate in a simple manner into similar increases in the amplitude of action potential-evoked responses. This assumption is rarely tested but is important in terms of understanding the impact of scaling on cellular or network function. However, this assumption could be inaccurate. For instance, if vesicle pools contributing to miniature and evoked release are not completely overlapping then changes in mEPSC amplitude may not be observed as similar changes in action potential-evoked current amplitude. In addition, a simple translation of mEPSC amplitude to evoked current amplitude could be more complicated if increases in mEPSCs amplitude trigger compensatory changes in probability of release. In order to determine the relationship between mEPSCs and evoked amplitudes, we triggered scaling in chick embryonic spinal motoneurons *in vivo*, measured evoked AMPAergic currents (eAMPA), and assessed probability of release (Pr) at glutamatergic terminals using the paired pulse protocol. We found that triggering upward AMPAergic scaling produced an increase in eAMPA amplitude without significant changes in Pr. These findings suggest that increases in quantal amplitude translate in a relatively straightforward manner into evoked glutamatergic synaptic strength. However, previously we have shown that the scaling mechanisms differ between glutamatergic and GABAergic synapses. Therefore, currently we are investigating if similar relationship between quantal amplitude and evoked synaptic strength occurs at GABAergic synapses.

Disclosures: D. Pekala: None. **P. Wenner:** None.

Poster

202. Homeostatic Synaptic Plasticity

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 202.22/B68

Topic: B.07. Synaptic Plasticity

Support: NIH Grant EY02858
NIH Grant MH071666
The Mathers Foundation

Title: Preservation of E-I balance despite enhanced OD plasticity and spine density in hippocampus and cortex of mice lacking PirB

Authors: *M. DJURISIC[1], A. JAGGI[2], C. J. SHATZ[1];
[1]Biology, Neurobiology, and Bio-X, [2]Biol., Stanford Univ., Stanford, CA

Abstract: During developmental critical periods, extensive synapse pruning takes place. The neuronal MHC class I receptor PirB is expressed at excitatory synapses, where it is required for pruning in visual cortex and hippocampus (Djurisic et al, 2013; 2018). We have reported that there is increased OD plasticity, better performance in cognitive tests, and higher density of excitatory synapses in both young and adult mice with either germline or conditional deletion of PirB in pyramidal neurons. These phenotypes can be explained by a concomitant absence of LTD and increased LTP at excitatory synapses, in both cortex and hippocampus of PirB null mice.

It has been suggested that an imbalance between excitation and inhibition is required for learning and plasticity. Since there are excessive numbers of excitatory synapses in cortex of mice lacking PirB, it is possible that the observed changes in plasticity and learning are due to an imbalance between excitation and inhibition. Here using patch clamp recordings from neurons in germline PirBKO mice, we find that the frequency of miniature excitatory postsynaptic currents (mEPSCs) is higher in pyramidal neurons in visual cortex and in CA1 hippocampal neurons. This increase is accompanied by an ~40% increase in the frequency of inhibitory postsynaptic currents (mIPSC) in L2/3 pyramidal neurons in visual cortex relative to WT cohorts. A similar increase in mIPSC frequency is also present in CA1 hippocampal neurons of PirBKO mice. Conditional deletion of PirB from CA1 pyramidal cells recapitulates higher mIPSC frequency, implying that PirB expression in CA1 neurons is required to regulate retrogradely inhibitory inputs. In L2/3 and L5/6 of cortex in PirBKO, there is also an increase in the density of perineuronal nets (PNNs), pointing to potential changes in inhibitory drive onto PV interneurons, as well as pyramidal cells.

Together these observations show that the deficient synapse pruning and resulting excess in excitatory synapses in neurons lacking PirB is accompanied by increased functional inhibition, and point to PirB negatively modulating both excitation and inhibition. Thus, despite increased plasticity and enhanced learning in mice lacking PirB, E-I balance appears to be preserved in both cortex and hippocampus.

Disclosures: **M. Djurisic:** None. **A. Jaggi:** None. **C.J. Shatz:** None.

Poster

202. Homeostatic Synaptic Plasticity

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 202.23/B69

Topic: B.07. Synaptic Plasticity

Support: UK Dementia Research Institute

Title: Age-related decline in homeostatic plasticity

Authors: *C. I. RADULESCU, N. ZABOURI, S. J. BARNES;
Dept. of Med., UK Dementia Res. Inst. at Imperial Col. London, London, United Kingdom

Abstract: Increased neuronal hyperactivity is a functional hallmark of healthy ageing and an early sign of pathology in Alzheimer's disease. The molecular and neural-circuit plasticity mechanisms that underpin the emergence of age-related hyperactivity remain unclear. Here, we use a combination of *in vivo* 2-photon calcium imaging and *ex vivo* slice electrophysiology to investigate the emergence of destabilised cortical activity in late adulthood. We measured the functional calcium related neuronal activity of superficial cortical neurons during early (2 m) and late (8 m) adulthood in mouse cortex. We find evidence for emerging hyper-activity in late adulthood that is specific to a sub-population of the most active neurons. We then explored the neural circuit origins of this selective hyperexcitability using *ex vivo* electrophysiological recordings of excitatory and inhibitory synaptic inputs to probe homeostatic plasticity driven by prolonged exposure to patterned LED stimulation (40 Hz light flicker, intermittently across 96 hours). We find, LED stimulation induced homeostatic down-scaling of synaptic responses in young, but not late adult mice, suggesting an age-related decline in homeostatic plasticity may account for the emergence of age-related neuronal hyperactivity.

Disclosures: C.I. Radulescu: None. **N. Zabouri:** None. **S.J. Barnes:** None.

Poster

202. Homeostatic Synaptic Plasticity

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 202.24/B70

Topic: B.07. Synaptic Plasticity

Support: NIH Grant DC015508

Title: Time course of activity dependent homeostasis at auditory nerve synapses

Authors: N. F. WONG, *M. A. XU-FRIEDMAN;
SUNY Buffalo, Buffalo, NY

Abstract: Abnormal levels of acoustic stimulation can result in hearing problems such as tinnitus and language processing disorders. It is important to understand how long it takes for changes in the acoustic environment to trigger lasting changes in auditory physiology. We

addressed this issue by using whole-cell patch clamp of bushy cells in slices of the cochlear nucleus from mice that were exposed to noise or had ligated ear canals. Presynaptic auditory nerve fibers were electrically stimulated in pairs of pulses and the ratio between EPSCs was used to assess synaptic depression. Previous work has shown that a week of high acoustic stimulation leads to an increased pool of releasable vesicles, and decreased synaptic depression. The opposite occurs after a week of decreased acoustic stimulation by occluding ears. We found that both EPSC amplitude and synaptic depression decreased after 18 hr of noise exposure and increased after 48 hr of ear canal ligation. Over the next 7 days EPSC amplitude gradually increased in noise-exposed animals and gradually decreased in ear-occluded animals to control levels. Together, these results suggest that changes to the probability of vesicle release happen rapidly followed by slower changes to the size of the pool of releasable vesicles. Furthermore, recovery was assessed in mice that were exposed to noise for 3 or 10 days and then returned to a quiet, control environment. Synaptic depression recovered within 12 hr after 3 days of noise exposure and within 24 hr after 10 days. Mice that were noise-exposed for 12 hr a day for 7 days exhibited decreased synaptic depression. These results indicate that auditory nerve synapses began to undergo important physiological changes rapidly after changes in acoustic environment and that these effects could accrue over time. This suggests it may be important to ameliorate abnormal acoustic activity quickly before cellular changes can take place.

Disclosures: **N.F. Wong:** None. **M.A. Xu-Friedman:** None.

Poster

202. Homeostatic Synaptic Plasticity

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 202.25/B71

Topic: B.07. Synaptic Plasticity

Title: The influence of sleep on the brain translatome

Authors: *J. L. SANTOS;
Univ. of Surrey, Guildford, United Kingdom

Abstract: Supervision: Julie Seibt; Co-Supervision: Andre Gerber
Background and project aims: Evidence suggest that sleep has various influences on cognition, in particular on the molecular mechanism underlying the long term consolidation of learning and memory. Memory is supported by mechanisms of synaptic plasticity, the cellular correlate of changes in communication between individual neurons. The consolidation of those memories are mediated by structural proteins that ultimately changes the morphology and biophysical properties of the synapses. Studies have shown that the long-term consolidation of synaptic changes are dependent on the local synaptic translation during sleep. The majority of sleep studies have focused on transcriptomics in the brain for plasticity processes. But whether sleep

uses synaptic translation and/or modifies the brain's translatome for plasticity processes has never been systematically assessed. The aim of this study is to quantify the rate of protein synthesis at cortical synapses across sleep and wakefulness and to identify the cortical translatome during sleep influenced by novel experience.
Methods and results: We used various behavioural manipulations to test the effect of sleep and experience on translation in the rodent cortex. To measure translation activation we used Surface sensing of translation (SUnSET) to identify the cortical translatome, we performed polysome profiling followed by RNA-sequencing. Our preliminary results show that phosphorylation levels of molecular translation initiator 4EBP1, was increased during sleep which supports our hypothesis that sleep enhances translation in the brain. Ongoing analysis aims now to characterise which mRNAs are translated during sleep after experience to better understand the molecular mechanisms at the basis of sleep-dependent brain plasticity. This work will shed the light on how sleep regulates global translation in the brain which ultimately impacts synaptic plasticity consolidation.

Disclosures: J.L. Santos: None.

Poster

202. Homeostatic Synaptic Plasticity

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 202.26/B72

Topic: B.07. Synaptic Plasticity

Support: NIH Grant MH117089
Mcknight Foundation Grant to M.X.
Whitehall Foundation Grant to M.X.

Title: Input-specific homeostatic synaptic plasticity in the mouse visual cortex

Authors: *Z.-L. CAI[1,2], M. SCANZIANI[3], M. XUE[1,2];
[1]Dept. of Neurosci., Baylor Col. of Med., Houston, TX; [2]The Cain Fndn. Labs., Jan and Dan Duncan Neurolog. Res. Inst. at Texas Children's Hosp., Houston, TX; [3]Univ. of California, San Francisco, San Francisco, CA

Abstract: Homeostatic synaptic plasticity is fundamental for the mammalian cerebral cortex to counteract perturbations and maintain stable functions. Dysfunction of homeostatic synaptic plasticity is increasingly linked to neurodevelopmental disorders such as epilepsy and autism. A number of homeostatic synaptic plasticity mechanisms have been discovered using different experimental preparations and paradigms. For example, synaptic scaling enhances all excitatory inputs onto a neuron in a multiplicative manner when its activity is reduced. However, it is still unclear if synaptic inputs originating from distinct presynaptic neurons and converging onto the

same neuron are differentially affected by homeostatic synaptic plasticity. Here we show that in the mouse visual cortex, reduction of neuronal excitability of individual neurons *in vivo* differentially affects their synaptic inputs, depending on the identities of presynaptic neurons. We overexpressed an inwardly rectifying potassium channel (Kir2.1) to reduce the excitability of a subset of layer 2/3 pyramidal neurons *in vivo*. We also expressed channelrhodopsins in different cell types for selective photostimulation of specific synaptic inputs. We found that the excitatory inputs from layer 5 and layer 6 were increased in Kir2.1-expressing neurons, whereas those from layer 2/3, layer 4, and the thalamus did not change. Our results indicate that homeostatic synaptic plasticity manifests in an input-specific manner *in vivo*. We anticipate that the input-specific homeostatic plasticity mechanisms identified here will have implications for understanding how brain plasticity copes with sensory perturbations or injuries.

Disclosures: Z. Cai: None. **M. Scanziani:** None. **M. Xue:** None.

Poster

202. Homeostatic Synaptic Plasticity

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 202.27/B73

Topic: B.07. Synaptic Plasticity

Support: NIH grants MH086403 (L.C.)
MH091193 (L.C.)
HD084215 (L.C.)
MH086403 (to T.C.S.)

Title: Synaptic retinoic acid receptor signaling mediates mTOR-dependent metaplasticity that controls hippocampal learning

Authors: *Y.-T. HSU[1], J. LI[1], D. WU[2], T. C. SUDHOF[2], L. CHEN[1];
[1]Stanford Inst. of Neuro Innovation and Translational Neurosci, [2]Stanford Univ., Stanford, CA

Abstract: Homeostatic synaptic plasticity is a stabilizing mechanism engaged by neural circuits in response to prolonged perturbation of network activity. The non-Hebbian nature of homeostatic synaptic plasticity is thought to contribute to network stability by preventing "runaway" Hebbian plasticity at individual synapses. However, whether blocking homeostatic synaptic plasticity indeed induces runaway Hebbian plasticity in an intact neural circuit has not been explored. Furthermore, how compromised homeostatic synaptic plasticity impacts animal learning remains unclear. Here, we show in mice that the experience of an enriched environment (EE) engaged homeostatic synaptic plasticity in hippocampal circuits, thereby reducing excitatory synaptic transmission. This process required RARα, a nuclear retinoic acid receptor that doubles as a cytoplasmic retinoic acid-induced postsynaptic regulator of protein synthesis.

Blocking RARα-dependent homeostatic synaptic plasticity during an EE experience by ablating RARα signaling induced runaway Hebbian plasticity, as evidenced by greatly enhanced long-term potentiation (LTP). As a consequence, RARα deletion in hippocampal circuits during an EE experience resulted in enhanced spatial learning but suppressed learning flexibility. In the absence of RARα, moreover, EE experience superactivated mammalian target of rapamycin (mTOR) signaling, causing a shift in protein translation that enhanced the expression levels of AMPA-type glutamate receptors. Treatment of mice with the mTOR inhibitor rapamycin during an EE experience not only restored normal AMPA-receptor expression levels but also reversed the increases in runaway Hebbian plasticity and learning after hippocampal RARα deletion. Thus, our findings reveal an RARα- and mTOR-dependent mechanism by which homeostatic plasticity controls Hebbian plasticity and learning.

Disclosures: **Y. Hsu:** None. **J. li:** None. **D. Wu:** None. **T.C. Sudhof:** None. **L. Chen:** None.

Poster

203. Mechanisms Underlying Seizure Development and Epilepsy

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 203.01/B74

Topic: B.10. Epilepsy

Support: Merit Review Award I01 BX002305

Title: Reducing glycolytic flux decreases epileptic activity in the CA3 region of the rat hippocampus

Authors: *Y.-Z. PAN[1], T. P. SUTULA[1], P. A. RUTECKI[1,2];
[1]Dept of Neurol., Univ. of Wisconsin, Madison, WI; [2]Dept of Neurol., William S. Middleton Mem. Veterans Hosp., Madison, WI

Abstract: 2-deoxy-D-glucose (2DG), which differs from glucose only by removal of an oxygen atom at the 2-position, competes with glucose for uptake through glucose transporters in response to cellular metabolic energy demand, and undergoes phosphorylation at the 6-position. 2DG inhibits glycolysis because 2-deoxy-D-glucose-6 phosphate cannot undergo isomerization to 2-deoxy-D-fructose-6-phosphate, thereby reducing glycolytic flux and subsequent steps of glycolysis. 2DG has anticonvulsant actions in both *in vivo* and *in vitro* models of seizures and epilepsy (Stafstrom et al., Ann Neur 65: 435, 2009; Shao et al., JNeurophysiol 118:103, 2017), and in our recent study 2DG reduced epileptic network activity in CA3 neurons in hippocampal slices by presynaptic mechanisms (Pan et al. JNeurophysiol 121:1092, 2019). If the anticonvulsant action of 2DG is a result of glycolytic inhibition, reducing glycolytic flux by lowering ambient glucose concentration should also have anticonvulsant effects. To test this possibility, the effects of varying bath concentrations of 2DG and glucose on extracellular CA3

network bursts evoked by exposure to 7.5 mM $[K^+]_o$ were systematically evaluated in transverse hippocampal slices from adult Sprague-Dawley rats. Brain slices lacking *in vivo* cerebrovascular autoregulation are typically bathed in artificial cerebral spinal fluid (ACSF) with 10 mM glucose to support diffusion-dependent delivery and achieve physiological glucose concentrations of 2.5 - 4.4 mM. Lowering glucose from 10mM to 5mM had no effect on rate of epileptiform bursts in CA3, but in 2.5 mM glucose network bursts were reduced by 33% ($p < 0.01$). In 10 mM glucose, 1mM 2DG had no effect but 2.5, 5, or 10 mM 2DG reduced the burst rate, respectively, by 13, 35, and 56% ($p < 0.05$, ANOVA). In 5 mM glucose which had no effect on network burst rate, 2.5, 5, or 10mM 2DG reduced the burst rate, respectively, by 16, 45, and 72% ($p < 0.05$, ANOVA). Effects of reduced ambient glucose and 2DG were synergistic. In 2.5 mM glucose which reduced burst rate by 33%, concentrations of 2.5 or 5 mM 2DG further reduced burst rate to 77 and 94% compared to controls ($p < 0.05$, ANOVA). Some slices became nonviable in conditions of 2.5 mM glucose and 10 mM 2DG, confirming the critical dependence of hippocampal slices on diffusion-driven delivery of glucose and glycolysis. The results demonstrate that reducing glycolytic flux is associated with anticonvulsant actions, and further confirm the anticonvulsant properties of 2DG. Studies are underway to determine whether reduction of burst rate in conditions of reduced ambient glucose (2.5 mM) is dependent on presynaptic mechanisms as observed during bath application of 10mM 2DG.

Disclosures: **Y. Pan:** None. **T.P. Sutula:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); T.P.S hold intellectual property related to 2DG. **P.A. Rutecki:** None.

Poster

203. Mechanisms Underlying Seizure Development and Epilepsy

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 203.02/B75

Topic: B.10. Epilepsy

Support: NIH Grant NS075245
NIH Grant AA021213

Title: Increasing expression of the delta subunit of the $GABA_A$ receptor in dentate granule cells alters expression of associated alpha 4 and gamma 2 subunits in a mouse model of epilepsy

Authors: Z. PENG[1], N. ZHANG[1], C. S. HUANG[1], M. WALLNER[2], *C. R. HOUSER[1];
[1]Neurobio., [2]Mol. and Med. Pharmacol., David Geffen Sch. of Med. at UCLA, Los Angeles, CA

Abstract: While multiple changes in $GABA_A$ receptors ($GABA_A$Rs) have been identified in animal models of epilepsy, a decrease in δ subunit expression is one of the most consistent. The nonsynaptic δ subunit is responsible for much of the tonic inhibition in dentate granule cells

(GC), and reductions in δ subunit-containing $GABA_AR$s could contribute to increased excitability of these neurons. The decrease in δ subunit labeling is particularly striking because expression of several other $GABA_AR$ subunits, including the α4 and γ2 subunits, is substantially increased in the same regions. We propose that a decrease in the δ subunit is a critical alteration and that increased expression of the α4 and γ2 subunits could be related to the decrease in δ subunit expression. The goals of this study were to determine if Cre-dependent viral transfection for the δ subunit in dentate GC at two weeks following pilocarpine-induced seizures would selectively increase δ subunit expression in GC and lead to associated decreases in the aberrant α4 and γ2 subunit labeling. Pilocarpine-induced seizures were elicited in DOCK10-Cre mice (generously provided by Dr. Susumu Tonegawa) that express Cre selectively in dentate GC. At two weeks following status epilepticus, mice were transfected unilaterally with a Cre-dependent viral vector to express the δ subunit in GC at rostral and caudal levels of the hippocampus. At 4-6 weeks following transfection, expression of δ, α4 and γ2 subunits was compared in pilocarpine-treated mice with or without unilateral transfection of the δ subunit, and saline-treated control mice. Results confirmed previous findings of decreased δ and increased α4 and γ2 subunit labeling in the non-transfected epileptic mice compared to controls. In transfected pilocarpine-treated mice, a substantial increase in δ subunit expression was observed on the transfected side. Importantly, the aberrant increase in both α4 and γ2 subunits was substantially less on the transfected side of the pilocarpine-treated mice compared to the non-transfected side, and the levels of labeling were similar to those in control mice. These findings suggest that Cre-dependent transfection of the δ subunit in dentate GC in an animal model of epilepsy not only increases δ subunit expression selectively in these neurons, but also normalizes expression of the α4 and γ2 subunits, consistent with proposed partnerships among these subunits. Subsequent studies will determine if the normal subcellular localization of these subunits is reestablished in δ subunit-transfected pilocarpine-treated mice and if such changes alter tonic inhibition and seizure activity in these mice.

Disclosures: Z. Peng: None. **N. Zhang:** None. **C.S. Huang:** None. **M. Wallner:** None. **C.R. Houser:** None.

Poster

203. Mechanisms Underlying Seizure Development and Epilepsy

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 203.03/B76

Topic: B.10. Epilepsy

Support: American Epilepsy Society Young Investigator Award (MAH)
Dravet Syndrome Foundation Postdoctoral Fellowship (JHC)

Title: Disrupted hippocampal synaptic plasticity in the Scn1b knockout model of Dravet syndrome

Authors: A. G. MCCONNELL, J. H. CHANCEY, *M. A. HOWARD;
Neurosci., Univ. of Texas at Austin, Austin, TX

Abstract: Dravet Syndrome (DS) is a genetic form of epilepsy caused by mutations in the *SCN1A* or *SCN1B* genes. DS is associated with frequent seizures, cognitive deficits, and a high mortality rate. *SCN1B* codes for β1, a protein that interacts with voltage-gated ion channels important for neuronal excitability and synaptic integration. Previous studies have shown that loss of β1 leads to hyperexcitability in some pyramidal neurons, while the interneurons studied were unaffected. Changes in neuronal excitability are often associated with alterations in synaptic plasticity, a cellular correlate of learning and memory. It is unclear how loss of β1 affects plasticity. Here, we used an *Scn1b* knock-out (KO) mouse model of DS, with wild-type (WT) littermates serving as controls, to examine the effects of loss of β1 on hippocampal synaptic plasticity. We hypothesized that alterations in neuronal excitability and synaptic properties in *Scn1b* KO mice would lead to changes in synaptic plasticity. We recorded field excitatory post-synaptic potentials (fEPSPs) from CA1 in acute hippocampal slices while stimulating Schaffer collateral axons, and used theta burst stimulation paradigms (TBS) to induce synaptic plasticity. We found that *Scn1b* KO mice have deficits in long-term potentiation (LTP). We also found that a TBS with a longer inter-burst interval induced robust long-term depression (LTD) in WT mice, but the same stimulation did not induce plasticity in KO mice. Recent data shows that *Scn1b* KO mice have a delay in GABAergic maturation. We examined how $GABA_A$ antagonism affects induction of LTP. In WT mice, as expected, we found that blockade of GABAergic inhibition increased the magnitude of LTP. However, blocking GABA receptors did not augment LTP in slices from KO mice. Together, our data show that loss of β1 leads to deficits in multiple forms of synaptic plasticity, including reductions in both LTP and LTD, and alterations in GABAergic modulation of plasticity. We hypothesize that this loss of plasticity may be an underlying cause of the cognitive deficits associated with DS.

Disclosures: A.G. McConnell: None. **J.H. Chancey:** None. **M.A. Howard:** None.

Poster

203. Mechanisms Underlying Seizure Development and Epilepsy

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 203.04/B77

Topic: B.10. Epilepsy

Title: Characteristics of synaptic vesicle glycoprotein 2A (SV2A) binding in rat brain and changes after status epilepticus: A novel approach to measure presynaptic plasticity

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Abstract: Synaptic vesicle glycoprotein 2A (SV2A) is a transmembrane protein localized in the presynaptic vesicle, that binds levetiracetam and probably regulates inhibitory neurotransmission in the hippocampus. A novel radiotracer, [^{11}C]UCB-J, that binds to the levetiracetam site with high affinity is used for *in vivo* imaging, thus representing an attractive opportunity to study epileptogenesis and presynaptic plasticity. The binding characteristics of [^{3}H]UCB-J in rat and human cortex was investigated by *in vitro* receptor autoradiography. Repeated saturation experiments revealed K_D values between 2 nM and 5 nM, and complete displacement with levetiracetam in both rat and man. Displacement experiments in rat cortex showed a K_i value of 33 ± 5 nM for UCB-J and more than 100-fold lower binding affinity for brivaracetam (3.6 ± 0.5 μM) and levetiracetam (14 ± 9.0 μM), respectively. To evaluate the potential of [^{3}H]UCB-J binding as a marker of presynaptic plasticity, we examined changes in UCB-J binding to SV2A over time in the cerebral cortex and the hippocampus of rats with status epilepticus induced by pilocarpine. A prominent (> 30% in cortex and > 50% in hippocampus) and highly significant ($P < 0.001$) reduction in binding was observed in both regions 10 days after pilocarpine. The decline occurred slightly faster in the cortex, compared to the hippocampus where changes were first detected 48 hours after pilocarpine. In both regions, binding returned to basal levels 12 weeks after pilocarpine. These data demonstrate that SV2A binding using novel radiotracers is a reliable method to detect presynaptic plasticity during epileptogenesis, and is likely to be used for demonstrating synaptic plasticity more generally.

Disclosures: J.D. Mikkelsen: None. **V. Fagerholt:** None. **R.S. Petersen:** None. **B.A. Pazarlar:** None. **S.C. Pedersen:** None. **M. Labrouzi:** None. **S. Ali:** None. **H.D. Hansen:** None. **L. Pinborg:** None. **C. Vermeiren:** A. Employment/Salary (full or part-time):; UCB Pharma. **J.P. Bankstahl:** None. **P. Bascuñana:** None. **G.M. Knudsen:** None.

Poster

203. Mechanisms Underlying Seizure Development and Epilepsy

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 203.05/B78

Topic: B.10. Epilepsy

Support: NIH R01NS069861
NIH R01NS097750

Title: Reclusive chandeliers: Dentate axo-axonic cells are functionally isolated early after experimental status epilepticus

Authors: *A. PRODDUTUR[1], A. GUPTA[1], J. G. FERNÁNDEZ[2], V. SANTHAKUMAR[3]; [1]Univ. of California Riverside, Riverside, CA; [2]Rutgers NJMS, Newark, NJ; [3]Pharmacology, Physiol. & Neurosci., New Jersey Med. Sch. Dept. of Pharmacol. and Physiol., Newark, NJ

Abstract: Inhibitory neurons of the hippocampal dentate gyrus are crucial in maintaining the functional "gate" that regulates the spread of network excitability in the trisynaptic circuit. Molecular layer interneurons are positioned to provide feed-forward inhibition to granule cells (GCs). Specifically, we find that a class of parvalbumin (PV)-positive Axo-Axonic cells (AAC) are located in the dentate inner molecular layer (IML) and regulate GC firing by synaptically inhibiting the GC axon initial segment. Circuit effects of AACs are likely distinct from PV-positive basket cells (BCs) which underlie somatic feed-forward and feedback inhibition of GCs. Here we use adult mice from both sexes expressing neuronal channelrhodopsin (ChR2-EYFP) and PV reporter to examine alterations in the intrinsic and synaptic physiology of AACs one week after pilocarpine-induced status epilepticus (SE). Experimental mice, age-matched saline-injected and naïve controls were obtained by crossing homozygous *ChR2-EYFP-loxP* and *Parvalbumin-cre* lines on C57Bl/6 background. Ex-vivo patch-clamp recordings were performed on 300μm hippocampal slices. AACs were identified as EYFP positive cells in the IML with characteristic axonal cartridges. Morphophysiologically distinct AACs in post-SE mice showed a significant decrease in firing rate in response to suprathreshold somatic current injection compared to controls. However, AAC active and passive properties such as input resistance and action potential threshold remained unaltered after SE. The frequency of spontaneous excitatory and inhibitory synaptic currents in AACs were decreased early after SE suggesting reduced excitatory and inhibitory drive to AACs after SE. We assessed functional output from AACs to GC using paired patch-clamp recordings and Polygon400 DMD to optically stimulate individual AACs expressing ChR2 in the IML by utilizing 3-5 ms pulses of blue light to evoke unitary inhibitory postsynaptic currents (uIPSC) in GCs. Although the reliability of AAC→GC synapses remained unchanged, there was an apparent reduction in uIPSC amplitude in GCs after SE which did not reach statistical significance. These findings suggest that AACs undergo a robust early decrease in excitability and receive fewer baseline excitatory and inhibitory synaptic inputs after SE. Such a functional disconnection after SE could impair feed-forward inhibition of dentate granule cells and compromise the dentate gate at a critical period of network plasticity early post seizures.

Disclosures: A. Proddutur: None. **A. Gupta:** None. **J.G. Fernández:** None. **V. Santhakumar:** None.

Poster

203. Mechanisms Underlying Seizure Development and Epilepsy

Location: Hall A

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Program #/Poster #: 203.06/B79

Topic: B.10. Epilepsy

Support: NIH Grant 5R01NS040109-1

Title: Characterization and functional role of neuronal chloride microdomains

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Abstract: Subcellular variation in the direction of Cl^- currents elicited by $GABA_A$ receptor activation have been reported for over 10 years, but the reasons for this variance remain controversial and poorly understood. We tested whether subcellular variance in cytoplasmic chloride concentrations ($[Cl^-]_i$) underlies these findings. We determined the local $[Cl^-]_i$ by 3 different highly sensitive methods: 1. Two-photon imaging of SuperClomeleon, a ratiometric Cl^--sensitive fluorophore consisting of two fluorescent proteins CFP and YFP, joined by a short polypeptide linker which allows FRET-based imaging. 2. Fluorescent Lifetime IMaging (FLIM) of a Cl^--sensitive dye, MEQ (6-methoxy-N-ethylquinolinium iodide) delivered to the cytoplasm via whole-cell recording pipette. Unlike SuperClomeleon, MEQ is insensitive to pH, while for both fluorophores the Cl^--sensitive signal is independent of the concentration of dye. 3. Electrophysiological measurements of the reversal potential of membrane currents elicited by local application of GABA (E_{GABA}) using whole-cell and gramicidin-perforated patch-clamp recordings and calculation of $[Cl^-]_i$ based on Nernst equation. All three methods show evidence of $[Cl^-]_i$ microdomains in dendrites of individual neurons. In addition, there are highly significant correlations between $[Cl^-]_i$ measured by fluorescent imaging and FLIM with $[Cl^-]_i$ calculated based on E_{GABA} and Nernst equation. Our data demonstrate that $[Cl^-]_i$ varies in different segments of dendrites, that the borders of these microdomains are highly stable over a course of an hour, and that stability is unaffected by pharmacological inhibition of cation-chloride cotransporters. These findings are novel and point to the presence of dendritic Cl^- microdomains that regulate the impact of GABAergic inputs. To determine whether the Cl^- microdomains participate in regulation of inhibitory synapses, we recorded single pyramidal cells by whole-cell patch clamping while stimulating different inhibitory interneurons by puffing glutamate to their cell bodies. There is a wide range in E_{GABA} at different interneuron-pyramidal cell synapses. These studies provide new insight into neuronal chloride homeostasis and the existence of functionally significant neuronal Cl^- microdomains.

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Poster

203. Mechanisms Underlying Seizure Development and Epilepsy

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 203.07/B80

Topic: B.10. Epilepsy

Title: Differential contributions from synaptic and extrasynaptic GABA-A receptors support dynamic shifts in circuit patterns during seizure evolution

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Abstract: GABA-ARs containing gamma2 subunits mediate phasic inhibitory postsynaptic currents (IPSCs) in hippocampal granule cells in response to brief high concentration transmitter release and rapidly desensitize to low-level tonic or brief hi-frequency pulsatile GABA exposure. Conversely, extrasynaptic GABA-ARs containing delta subunits largely are non-desensitizing, have greater GABA affinity, and are responsible for tonic inhibitory currents in response to mostly stable low concentrations of extracellular GABA, but also detect synaptic ‘spillover’. With convulsant stimulation and seizure initiation, a loss of synaptic inhibition occurs and an increase in extracellular GABA (1-3 uM) is inferred from tonic current measurements. Receptor kinetic computational models of GABA-ARs optimized to fits of synaptic IPSCs, extrasynaptic tonic currents, and multisynaptic evoked IPSCs were used to simulate GABAergic system responses under different circuit conditions. Synaptic receptors rapidly desensitize with nearly a 90% loss of inhibition at 160 Hz after only 100 msec, simulating the effects of epileptic ‘fast ripples’. Recovery from both ‘fast’ and ‘slow’ desensitized receptor states occurs by 10 sec, but superimposed lower frequency activity (0.5 - 2 Hz) and/or low level GABA (< 1uM) sustains the loss of synaptic inhibition by maintaining a significant proportion of postsynaptic GABA-ARs in desensitized states. Composite models including synaptic and extrasynaptic GABA-ARs show that hi-frequency stimulation promotes GABA spillover, and only a few extrasynaptic delta subunit-containing receptors (~ 4 per synapse vs. 36 postsynaptic gamma subunit-containing receptors per synapse) account for up to 60% of the charge transfer of an evoked inhibitory response, prolonging and broadening the spatial extent of synaptically-released GABA and favoring network slowing. At a frequency of 3-6 Hz, 10-20 % of synaptic GABA-ARs remain desensitized sustaining a loss of inhibition while spillover to extrasynaptic receptors supports a synchronous oscillatory response. In summary, evidence is provided supporting a mechanism of seizure evolution from an initiation phase of fast-rhythmic activity and loss of synaptic inhibition that progresses to slowed synchrony. This involves a dynamic shift of activation from synaptic to

extrasynaptic GABA-ARs, and further analysis will determine whether increases in tonic extracellular GABA arrest this clonic phase and terminate the seizure with post-ictal depression.

Disclosures: D.E. Naylor: None.

Poster

203. Mechanisms Underlying Seizure Development and Epilepsy

Location: Hall A

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Program #/Poster #: 203.08/B81

Topic: B.10. Epilepsy

Support: EpiBiosS4Rx Grant/5U54NS100064
CONACYT Scholarship/377478

Title: Spontaneous alterations in EEG spike patterns acutely after experimental traumatic brain injury

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Abstract: Traumatic brain injury (TBI) is a serious health problem and about 50% of severe TBI cases will develop post-traumatic epilepsy (PTE) in their lifetime. Currently, there are no treatments to prevent PTE, making the discovery of new treatments greatly beneficial for understanding the mechanisms associated with PTE development. A common rat model used to study PTE is lateral fluid percussion injury (LFPI) and this model, like other acquired and genetic models of epilepsy generates spontaneous epileptic seizures. However, these models as well as sham rats also generate other spontaneous electrographic patterns such as isolated or bursts of EEG spikes without or with slow waves (e.g. spike-wave discharges) in the neocortex and hippocampus. It is unclear whether EEG spikes and the various spiking patterns play a role in the development of late posttraumatic seizures. To begin to address these questions, the current study focused on quantifying the EEG spikes and their features during the acute period after injury induced by LFPI in adult, male Sprague Dawley rats (n=19) over the left hemisphere using a fluid-percussion device. During the same surgery session, electrodes were implanted within the cortex and hippocampus ipsilateral to the injury, and in the cortex contralateral to the injury. A second cohort of rats (“sham”; n=6) underwent the same surgical procedures as the TBI rats, but TBI was not induced. The electrodes were mounted in 12-hole Plastic 1 pedestal and connected to the input. EEG activity was monitored 24/7 for the first week after TBI, sampled at 2 kHz/channel and with bandpass settings of 0.1-500 Hz. Results showed in rats that survived severe TBI (13 out of 19), there was a low percentage (8%) of rats that had complications from implanting electrodes immediately after TBI, whereas all of the sham rats survived without

complications. Review of the EEG recorded for the first 7 days found acute cortical and hippocampal EEG spikes in all the animals. EEG spikes were detected each day for 7 days in the TBI rats, but in sham rats they were found for only the first 2-3 days after surgery. Furthermore, in the TBI rats, EEG spikes appeared earlier after surgery (4.9 ± 3.6 vs 26.2 ± 2.1 hr), with a lower spectral frequency (2-4 vs 5-11 Hz), and with a longer duration (32 ± 14 s vs 7 ± 3 s) than those in sham rats. These data indicate that TBI alters neuronal circuity that generates EEG spikes. Ongoing EEG recording and analysis will quantify the proportion of TBI rats with chronic posttraumatic seizures to determine whether EEG spike disturbances are associated with the development of PTE.

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Poster

203. Mechanisms Underlying Seizure Development and Epilepsy

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 203.09/B82

Topic: B.10. Epilepsy

Support: R01 NS083402
R01 NS097610

Title: Sex differences in KA-induced seizure propensity by genetic and pharmacological inhibition of brain-specific tyrosine phosphatase STEP

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Abstract: STriatal Enriched protein tyrosine Phosphatase (STEP) is a brain-specific tyrosine phosphatase. Membrane-bound $STEP_{61}$ isoform dephosphorylates and internalizes ionotropic glutamate receptors including NMDA and AMPA receptors. High levels of $STEP_{61}$ have been detected in human post-mortem tissue and animal models of Alzheimer's disease (AD), which display hippocampal hyperexcitability as one comorbidity. In addition, genetic knock-out of STEP or its pharmacological inhibition alleviates excitatory synaptic deficits and memory loss observed in AD mouse models. However, the roles of $STEP_{61}$ on seizures and hippocampal network hyperexcitability are not clear. Since $STEP_{61}$ is a key negative modulator of synaptic strengthening in the HP, we hypothesized that inhibition of STEP would increase seizure susceptibility to chemoconvulsant. To test this, we injected STEP KO mice and their wild-type (WT) littermates with kainic acid (KA, 30 mg/kg, i.p) or saline (control, i.p.), and scored their behavioral seizures using Racine scale. For pharmacological inhibition, WT mice were injected

with TC-2153 (10 mg/kgor vehicle control 3 hours before KA injection. STEP KO mice showed reduced cumulative seizure scores compared to WT littermates. TC-2153-injected WT males showed significant decrease in seizure propensity compared to control-injected males. Interestingly, TC-2153-injected females displayed larger decrease in seizure propensity. These data indicate that both genetic deletion and pharmacological inhibition of $STEP_{61}$ in the hippocampus reduces KA-induced seizures. Next, we examined the expression of known STEP substrates and β-amyloid (Aβ) oligomers after KA-induced SE since we have previously shown that hippocampal Aβ and $STEP_{61}$ expression are increased in response to heightened hippocampal neuronal activity in rats (Jang et al., 2016). We found that KA-induced seizures elevate expression of Aβ, whereas such increase was abolished in STEP knock-out mice. We are currently investigating how Aβ level is upregulated in a STEP-dependent manner and whether such regulation mediates KA-induced SE associated via increasing hippocampal network hyperexcitability.

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Poster

203. Mechanisms Underlying Seizure Development and Epilepsy

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 203.10/B83

Topic: B.09. Network interactions

Title: Variability in seizure phenotypes in individual rats with epilepsy

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Abstract: A classification of seizures with focal onset based on dynamics, in particular onset and offset patterns, has been proposed (Jirsa, et al. 2014). Among the 16 possible types, the saddle node/homoclinic (SN/SH), seems to be predominant across species (from humans to zebra fish). Based on a general theory of bursting dynamics (Saggio et al. 2017), we predicted that individuals with epilepsy may navigate the map of brain dynamics and express different types of seizures. We tested this hypothesis in an experimental model of temporal lobe epilepsy. We enrolled 10 male Sprague Dawley rats treated with pilocarpine (380 mg/kg, i.p.), which developed spontaneous epileptic seizures (Toyoda et al., 2015). Electroencephaogram (EEG) was obtained with a Microdrive (NLX 9-drive, Neuralynx) with bandpass filter (0.1-1800 Hz) and sampling rate 2 kHz. In each seizure, the seizure class was established based upon the identification of the type onset (SN, SNIC, SupH, or SubH) and offset (SH, SNIC SupH, or

FLC). We evaluated i) the seizure duration, ii) the amplitude of the DC shift that characterizes SN onset, iii) the number of brain regions involved during seizure propagation, iv) the number of brain regions characterized by high fast ripple (FR: 300-600 Hz) power, and v) the propagation time to the thalamus. In line with our hypothesis, we confirmed the existence of several types of seizures in each individual animal. The duration of seizures with SN onset tended to be shorter than that starting with another type (in 5 out of 8 rats). For SN onset seizures, the abnormal activity easily propagated to surrounding area (in 4 out of 8 rats). However, there was no relationship between the type of seizure and the number of brain regions displaying high FR power (in 7 out of 8 rats). Finally, seizures with a large DC shift at onset were characterized by a smaller number of electrodes with high FR power (in 5 out of 8 rats). In this rat model, all animals display several types of seizures, which may contribute to their drug-resistance. The most frequent form of seizures starting with a DC shift (SN onset) is characterized by faster propagation to surrounding regions possibly involving the thalamus.

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Poster

203. Mechanisms Underlying Seizure Development and Epilepsy

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Program #/Poster #: 203.11/B84

Topic: B.10. Epilepsy

Support: NIH grant 1R01NS092705
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AES 506835

Title: The potassium channel Kv4.2 regulates dendritic morphology, seizure susceptibility, and electrographic dynamics in mice

Authors: *D. TIWARI[1], T. L. SCHAEFER[2], L. M. SCHROEDER-CARTER[1], J. M. KRZESKI[1], A. T. BUNK[1], A. SNIDER[1], R. DANZER[1], M. T. WILLIAMS[1,3], C. V. VORHEES[1,3], S. C. DANZER[5,3,4], C. GROSS[1,3];
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Abstract: The voltage-gated potassium channel Kv4.2 is a critical regulator of dendritic excitability in the hippocampus, and is crucial for dendritic signal integration. Kv4.2 mRNA and protein expression as well as function are reduced in several genetic and pharmacologically induced rodent models of epilepsy and autism. It is not known, however, if reduced Kv4.2 is just

an epiphenomenon of epilepsy and autism, or if it causes neuronal hyperexcitability and autistic-like behavior. In the present study, we used Kv4.2 heterozygous mice and adult-onset manipulation of hippocampal Kv4.2 expression in mice to assess Kv4.2's role in regulating electrographic dynamics**,** seizure susceptibility, anxiety, sensory-motor gating related behaviors, fear conditioning and neuronal morphology in the brain. Male mice were used for all experiments, except for the analysis of dendritic spine morphology, for which both female and male mice were used. Littermate controls were used in all experiments, and the sample size ranged between 5 and 18 per condition. We observed an overall reduction in dendritic spine density and reduced proportions of mushroom, but increased proportions of filopodia-like spines in both male and female Kv4.2 heterozygous mice compared to their sex-matched wild type littermates. No changes in anxiety or sensory motor gating behavior or retention of fear memory were detected in Kv4.2 heterozygous mice compared to littermates. Using EEG analyses, we show that reduction in Kv4.2 levels led to increased theta waveform power and increased occurrence of electrographic spikes. In line with increased neuronal excitability, the latency to onset of kainic acid-induced seizures was significantly shortened in Kv4.2 heterozygous mice compared to wildtype littermates, which was accompanied by a significant increase in theta waveform power. By contrast, intrahippocampal CA1 injection of lentivirus overexpressing Kv4.2 delayed seizure onset and reduced theta waveform power. Overall, these results show that Kv4.2 expression levels regulate seizure onset and severity but not anxiety-related behavior in male mice. In the future, manipulation of Kv4.2 expression and function can be used to alter seizure susceptibility in epilepsy.

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Poster

203. Mechanisms Underlying Seizure Development and Epilepsy

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 203.12/B85

Topic: B.10. Epilepsy

Support: National Science Centre, Poland (2016/20/S/NZ7/00424)

Title: Effects of TC-G 1008, GPR39 (zinc receptor) agonist in pentylenetetrazole-induced seizures and epilepsy

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Oslo, Oslo, Norway; [3]Dept. of Pharmacokinetics and Physical Pharm., Jagiellonian Univ. Med. Col., Krakow, Poland

Abstract: Motivation: Evidence from both preclinical and clinical studies suggest the importance of zinc homeostasis in diseases of the central nervous system which have been characterized by a defective balance between excitation and inhibition, such as epilepsy. The importance of extracellular zinc signaling via the G-protein coupled receptor 39 (GPR39) in inhibitory neurotransmission has been proposed by Chorin et al. (2011), thus suggesting GPR39 as a novel target for dampening seizures. To explore the possible role of this receptor in seizures and epilepsy we have previously assessed the effects of GPR39 activation with the aid of its potent and selective agonist, TC-G 1008, in various seizure and epilepsy models in mice. Here, we examined the effects of GPR39 agonist on pentyleneterazole (PTZ)-induced seizures in zebrafish larvae. Furthermore, we examined the impact of dietary zinc on the effects of TC-G 1008 in PTZ kindling model of epilepsy. **Methods**: 7-day old zebrafish larvae (approval no 15649) or male Albino Swiss mice (bodyweight range 25-30 g) (approval no 38/2017) were used. Larvae were pre-incubated with TC-G 1008 (70 µM) or $ZnCl_2$ (65 µM) for 20 h, and subsequently exposed to vehicle or PTZ (20 mM) for 5 min, and EEG was recorded. The mice were fed with a zinc adequate diet (ZnA) of 50 mg Zn/kg or a zinc deficient diet (ZnD) of 3 mg Zn/kg for 4 weeks. Following 4 weeks of the ZnA or ZnD, PTZ kindling was initiated. The ZnA and ZnD groups were subdivided into groups that received TC-G 1008 (10 mg/kg); zinc chloride ($ZnCl_2$) (8 mg Zn/kg); valproic acid (VPA) (150 mg/kg), a standard antiseizure drug; or vehicle, 0.5 h i.p (based on previous pharmacokinetic study) before each PTZ injection. ZnA or ZnD administration was continued during kindling. Seizures were characterized according to the modified Racine scale. **Results**: Pre-incubation of larvae with TC-G 1008 or $ZnCl_2$ did not significantly affect PTZ-induced changes in EEG, which is consistent with our data obtained in acute i.v. PTZ seizure threshold test in mice. In the PTZ kindling in mice, there was a significant effect of drug (TC-G 1008, VPA) and time on maximum seizure score (SS). The effect of the diet on SS was not significant. % of mice that exhibited at least 3 stage 4/5 seizures (fully kindled mice) was: 91.66% of mice that received TC-G 1008 + ZnA; 77.8% of TC-G 1008 + ZnD; 12.5% of $ZnCl_2$ + ZnA; 33% of $ZnCl_2$ + ZnD; 0% of VPA + ZnA; 25 % of VPA + ZnD; 46.15% of vehicle + ZnA; 58% of vehicle + ZnD. **Conclusions**: GPR39 activation may facilitate PTZ-induced epileptogenesis. These results, together with data obtained by our group and others, point to the complex role of zinc signaling with regard to seizures/epilepsy.

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Poster

203. Mechanisms Underlying Seizure Development and Epilepsy

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 203.13/B86

Topic: B.06. Synaptic Transmission

Support: AES Young Investigator Award
Dravet Syndrome Foundation Postdoctoral Fellowship
University of Texas at Austin

Title: Enhanced intrinsic excitability and synaptic integration in CA1 pyramidal cells in the SCN1B mouse model of Dravet syndrome

Authors: *J. H. CHANCEY, M. A. HOWARD;
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Abstract: Dravet syndrome (DS) is a genetic encephalopathy associated with severe, frequent seizures, developmental delays, cognitive deficits, and a high mortality rate. DS has limited treatment options, in part due to our lack of understanding of cellular and circuit level disruptions underlying the phenotype. Mutations in *SCN1B*, which encodes the voltage-gated ion channel auxiliary β1 subunit, have been linked to DS. β1 associates with and modifies the actions of ion channels regulating action potential initiation and dendritic excitability, such as $Na_v1.1$ and $K_v4.2$. Our goal is to investigate how loss of β1 changes synaptic/intrinsic interactions in hippocampal neurons to better understand the neurophysiological changes leading to the complex phenotypes of DS. Here we performed whole cell patch clamp recordings from CA1 pyramidal cells (PCs) in acute hippocampal slices from *Scn1b* knockout (KO) mice and wild-type (WT) littermates. Our data show that CA1 PCs from KOs are hyperexcitable, firing more spikes in response to current injection, without changes in rheobase, threshold, or resting membrane potential. KO neurons also exhibit modest increases in input resistance and I_h, and reduced capacitance. Additionally, we found complex changes in synaptic transmission. Using voltage clamp recordings and electrical stimulation of Schaffer collateral (SC) axons, we found smaller excitatory and inhibitory post-synaptic currents in KO PCs relative to WT. Despite this, SC stimulation evoked larger and prolonged depolarizations in KO PCs in current clamp. Temporal summation of SC inputs was enhanced in KO neurons. Both probability of firing and spike rate were increased in response to physiologically patterned theta burst stimuli. Similarly, spatial summation between SC and temporoammonic (TA) inputs was also enhanced. Synchronous theta burst stimulation of SC and TA inputs led to sublinear summation in WT PCs, but supralinear responses and increased firing in PCs from KO mice. Together our data suggest that modest changes in intrinsic excitability and synaptic properties lead to great enhancement of synaptic integration and input/output functions in *Scn1b* KO neurons, thereby fundamentally altering network excitability and control of information processing in the hippocampus.

Disclosures: J.H. Chancey: None. **M.A. Howard:** None.

Poster

203. Mechanisms Underlying Seizure Development and Epilepsy

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Topic: B.10. Epilepsy

Support: NINDS Grant 1U54NS079202

Title: Intramuscular administration of JNJ-55511118, a selective TARP-γ8 dependent AMPA receptor blocker, in the management of diisopropylfluorophosphate (DFP)-induced refractory status epilepticus in rats

Authors: *A. DHIR, M. A. ROGAWSKI;
Neurology, Sch. of Med., Univ. of California, Davis, Sacramento, CA

Abstract: Organophosphate (OP) intoxication may lead to benzodiazepine-refractory status epilepticus (SE). Because excessive glutamatergic neurotransmission is believed to contribute to OP-induced SE, AMPA-type glutamate receptors, the main mediators of glutamate-induced synaptic transmission, are potential treatment targets. Recently, JNJ-55511118 has been identified as a selective inhibitor of AMPA receptors containing the transmembrane AMPA receptor regulatory protein (TARP)-γ8 auxiliary subunit. TARP-γ8 is expressed throughout the hippocampus and other forebrain structures but there is little expression elsewhere in the brain. Accordingly, selective TARP-γ8 dependent AMPA receptor antagonists like JNJ-55511118 have been found to exhibit antiseizure activity in certain seizure models that are dependent upon forebrain involvement but in comparison with nonselective AMPA receptor antagonist such as perampanel TARP-γ8 agents have markedly reduced propensity to induce motor impairing side effects. In the present study, we sought to compare JNJ-55511118 and perampanel for their abilities to terminate seizures induced by the OP diisopropyl fluorophosphate (DFP). In preliminary studies in the mouse pentylenetetrazol seizure test, JNJ-55511118 significantly increased the threshold for clonus at doses of 10 and 20 mg/kg, IM without motor impairment. We further explored the effect of JNJ-55511118 in DFP-induced refractory SE. Male SD rats were implanted with 6-cortical screw electrodes for video-EEG recording and monitoring of seizures. DFP (4 mg/kg, SC) was administered to these rats followed 1-min later by atropine sulfate (2 mg/kg, IM) and pralidoxime chloride (25 mg/kg, IM) to avoid peripheral side effects. Forty minutes after DFP, animals were injected with midazolam (1.8 mg/kg, IM) to simulate the treatment that would be administered according to the standard clinical care protocol. Administration of midazolam at this time does not terminate DFP-induced high amplitude and high-frequency epileptiform activity. Immediately following midazolam, either JNJ-55511118 (5 mg/kg, IM) or perampanel (2 mg/kg, IM) was administered. JNJ-55511118 rapidly terminated epileptiform activity whereas perampanel also led to the termination of ongoing electrographic

seizures but the time required was greater (mean termination times 22 ± 9 min and 109 ± 18 min, respectively). In conclusion, both the nonselective AMPA receptor antagonist perampanel and the TARP-γ8 dependent AMPA receptor antagonist JNJ-55511118 terminated midazolam-refractory DFP seizures but the onset of action of JNJ-55511118 was markedly faster.

Disclosures: A. Dhir: None. **M.A. Rogawski:** None.

Poster

203. Mechanisms Underlying Seizure Development and Epilepsy

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 203.15/B88

Topic: B.10. Epilepsy

Title: Superbursts: Investigation of abnormal paroxysmal bursting activity in neuronal networks *in vitro*

Authors: *E. A. ROGERS[1], N. SURI[2], R. SHI[3];
[1]BME/BMS, Purdue, West Lafayette, IN; [2]Dept. of Med., Loyola Univ. Med. Ctr., Lombard, IL; [3]Depat. Basic Med. Sci., Purdue Univ., West Lafayette, IN

Abstract: The application of neuronal network dynamics to pharmacology requires temporally stable electrophysiological activity. Superbursts (SBs) are large, seemingly spontaneous activity fluctuations often encountered in high density neuronal networks in vitro. Little effort has been put forth to define and analyze SBs which are paroxysmal bursting discharges. Through qualitative and quantitative means, I have described specific occurrences of superbursting activity. A complex of paroxysmal bursting has been termed a "superburst episode," and each individual SB is a "superburst event" which is comprised of a fine burst structure. Quantitative calculations (employing overall spike summations and coefficient of variation (CV) calculations), reveal three distinct phases. Phase 1 is a "build up" phase of increasingly strong, coordinated bursting with an average of a 17.6% 13.7 increase in activity from reference. Phase 2, the "paroxysmal" phase, is comprised of massive coordinated bursting with high frequency spike content. Individual spike activity increases by 52.9% 14.6. Phase 3 is a "recovery phase" of lower coordination and an average of a 50.1% 35.6 decrease in spike production from reference. SBs can be induced and terminated by physical manipulation of the medium. Using a peristaltic pump with a flow rate of 0.4m1/min, superbursting activity ceases approximately 28.3 min after the introduction of flow. Alternatively, upon cessation of medium flow superbursting activity reemerges after approximately 8.5 min. Additionally, this study explored other methods capable of inducing superbursting activity using osmotic shocks. The induction and termination of SBs demonstrates that the cell culture environment plays a major role in generating this phenomenon. The observations that high density multi-layer neuronal networks in culture are more likely to enter paroxysmal bursting also supports the hypothesis that enrichment and depletion layers of

metabolites and ionic species are involved in such unusual activity. The dynamic similarity of the SB phenomenon with epileptiform discharges make further quantification on the spike pattern level pertinent and important.

Disclosures: **E.A. Rogers:** None. **N. Suri:** None. **R. Shi:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurovigor.

Poster

203. Mechanisms Underlying Seizure Development and Epilepsy

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 203.16/B89

Topic: B.10. Epilepsy

Support: NIH/NINDS 7R01NS036692-16
NIH/NINDS 7R01NS082851-04

Title: Assessment of extracellular matrix in mouse models of acquired epilepsy

Authors: *D. C. PATEL[1], N. SWIFT[2], B. P. TEWARI[1], L. CHAUNSALI[1], H. SONTHEIMER[3];
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Abstract: Epilepsy is a neurological disorder manifested by seizures which are caused by abnormal synchronous firing of population of neurons. Neurons and glia are two major components of the CNS and their tightly interwoven functions optimize neuronal network output. The molecular mechanisms that disrupt neuronal and/or glial functions and therefore leading to the development of epilepsy have been the prime area of research. Neurons and glial cells are embedded in a glue-like extracellular matrix (ECM) which is the third major component of the CNS and forms amorphous interstitial matrix and well-organized lattice-like structures called perineuronal nets (PNNs) encapsulating mainly neuronal soma. The ECM fills up small extracellular space (ECS) that surrounds all cellular structure and contributes to the maintenance of ionic distribution and transport of neurotransmitters, neuromodulators, nutrients and metabolites. The ECM is dynamic and the changes in its composition and expression level have been reported to regulate synaptic plasticity and to contribute to tissue remodeling after CNS injury. Degradation of ECM reduces the volume of the ECS and the studies in animals have shown positive correlation between reduced ECS and epileptiform activity. Some studies have reported changes in the ECM components in epileptic animals and human, however, a detailed characterization of ECM is lacking. In the present study, we investigated changes in the expression of ECM components in two clinically relevant mouse models of acquired epilepsy,

namely a viral infection and a chemoconvulsant-induced model of temporal lobe epilepsy. Mice were treated with either Theiler's murine encephalomyelitis virus (TMEV) or pilocarpine and the brains samples were collected during seizure periods to evaluate expression of PNNs. As PNN markers we used Wisteria floribunda agglutinin and hyaluronic acid binding proteins which bind to different components of PNN, namely chondroitin sulfate proteoglycans and hyaluronan, respectively. We found significant degradation of PNNs in the hippocampus of mice with seizures. Since PNNs are subject to digestion by proteolytic enzymes such as matrix metalloproteases (MMPs) and since levels of MMPs have been shown elevated in epileptic tissues, we also performed zymography and found a significant increase in the activity of MMPs in the hippocampus of mice with TMEV-induced seizures. These data suggest that changes in the level of ECM could be a result of increased activity of MMPs, which may contribute to the development of seizures. Further studies are directed to investigate causal relationship between changes in the ECM and development of epilepsy.

Disclosures: D.C. Patel: None. **N. Swift:** None. **B.P. Tewari:** None. **L. Chaunsali:** None. **H. Sontheimer:** None.

Poster

203. Mechanisms Underlying Seizure Development and Epilepsy

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Program #/Poster #: 203.17/B90

Topic: B.10. Epilepsy

Support: NIH grant NS047718
Craig H. Neilson Foundation
generous donations from Cure Medical
Research for Cure
J. Yonan was the recipient of a predoctoral fellowship from NIH 5T32 NS045540

Title: Vector-mediated pten deletion in adult dentate granule cells triggers *de novo* neuronal growth without apparent adverse consequences

Authors: *J. M. YONAN, K. M. YEE, O. STEWARD;
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Abstract: Phosphatase and tensin homolog (PTEN) is an important negative regulator of the mechanistic target of rapamycin (mTOR) pathway responsible for cell growth during development. PTEN has also been identified as a key negative regulator of axon regeneration in mature neurons because deletion or knockdown of PTEN in mature neurons enables robust regeneration of CNS axons and enhances axon sprouting. We have recently documented that PTEN deletion in uninjured adult neurons triggers *de novo* growth of neuronal cell bodies and

dendrites that continues for months. Still, the functional consequences of PTEN deletion, persistent mTOR activation, and *de novo* growth of mature neurons in the adult brain are not fully understood. Embryonic and early postnatal PTEN deletion results in brain hypertrophy and seizures, and promotor-driven PTEN deletion in adult-born granule cells in the dentate gyrus leads to spontaneous seizures and early death. Here, we report that AAV-mediated PTEN deletion in the dentate gyrus of mature rats and mice triggers *de novo* growth of cell bodies and dendrites without apparent adverse consequences. PTEN was deleted/knocked down in the dorsal dentate gyrus by injecting AAV/Cre into $PTEN^{f/f}$;$Rosa^{tdTomato}$ mice or AAVshPTEN into rats. With both approaches, immunostaining revealed complete absence of PTEN protein in the area of the injection with a surrounding penumbra of reduced expression. In the area of PTEN deletion, there were dramatic increases in the size of dentate granule cell bodies and nuclei and increases in the thickness of the granule cell layer. Counts of granule cells revealed no increases in granule cell number. There were also increases in the thickness of the molecular layer implying dendritic growth. Immunostaining for mossy fibers revealed no evidence of supra-granular mossy fibers, which develop following promoter-driven PTEN deletion in adult-born granule cells. Immunostaining for GAD67 revealed decreases in the density of GAD-positive terminals in the expanded granule cell body layer in the area of PTEN deletion. Immunostaining for tri-methylated H3K4 and H3K9 (markers for chromatin organization) revealed altered staining in PTEN-negative granule cells indicating a less condensed state of chromatin, suggestive of increased gene expression. Spontaneous seizures were not seen in either rats or mice (video monitoring is ongoing) and no animals died prematurely. The absence of mossy fiber sprouting and spontaneous seizures indicate that AAV-mediated PTEN deletion has different functional consequences than promoter-driven deletion in adult-born granule cells.

Disclosures: **J.M. Yonan:** None. **K.M. Yee:** None. **O. Steward:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); OS is a co-founder and holds economic interests in the company “Axonis”, which holds a license on patents relating to PTEN deletion and axon regeneration..

Poster

203. Mechanisms Underlying Seizure Development and Epilepsy

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 203.18/B91

Topic: B.10. Epilepsy

Support: NIH Grant NS088776

Title: A vitamin D enriched diet attenuates sex-specific behavioral deficits in the NS-Pten knockout mouse

Authors: *P. D. WOMBLE[1], S. L. HODGES[2], S. O. NOLAN[1], M. BINDER[1], A. HOLLEY[1], R. HERRERA[1], S. SENGER[1], D. JONES[1], E. KWOK[1], C. J. HERNANDEZ-ZEGADA[1], J. N. LUGO, JR[1];
[1]Psychology and Neurosci., [2]Inst. of Biomed. Studies, Baylor Univ., Waco, TX

Abstract: Among the various behavioral and physiological comorbidities, individuals with epilepsy are at a high risk for bone fractures (independent of seizure-related falls), as well as an increased likelihood of a comorbid diagnosis of Autism spectrum disorder (ASD). There is also evidence that individuals with epilepsy are deficient in vitamin D levels. The increase in bone fractures and decrease in vitamin D were hypothesized to be related to anti-seizure medications, however, there is a lack of consensus between studies. The neural subset-specific (NS) *Pten* knockout mouse has been well-characterized to show autistic-like deficits and have lower bone mineral density. This current study examined the effect of a vitamin D enriched diet in the NS-*Pten* knockout mouse. Mice were placed onto a vitamin D enriched diet starting at 4 weeks of age then behavioral testing began at 6 weeks of age. Behavioral testing included tests for general activity, anxiety, repetitive behaviors, social behaviors, and memory. Results indicated that a vitamin D diet attenuated hyperactivity exhibited by the male knockout mice ($p < 0.05$). In the elevated plus maze task, knockout males showed an increase in velocity, which was attenuated by the vitamin D diet ($p < 0.05$). In a social partition task, male and female knockout animals exhibited a reduction in sociability, however in male wildtype mice, vitamin D increased sociability ($p < 0.05$). Overall, these findings suggest that a vitamin D enriched diet had a significant impact on the behavioral phenotype of NS-*Pten* knockout mice.

Disclosures: P.D. Womble: None. **S.L. Hodges:** None. **S.O. Nolan:** None. **M. Binder:** None. **A. Holley:** None. **R. Herrera:** None. **S. Senger:** None. **D. Jones:** None. **E. Kwok:** None. **C.J. Hernandez-Zegada:** None. **J.N. Lugo:** None.

Poster

203. Mechanisms Underlying Seizure Development and Epilepsy

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 203.19/B92

Topic: B.10. Epilepsy

Support: NIH Grant R01NS065020, SCD
NIH Grant R01NS062806, SCD
NIH Grant F32NS083239, CLL

Title: Pten knockout cell load impacts dentate circuit abnormalities in a model of temporal lobe epilepsy

Authors: *C. L. LASARGE[1], R. Y. PUN[1], S. C. DANZER[1,2];
[1]Dept. of Anesthesia, Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [2]Departments of Anesthesia and Pediatrics, Univ. of Cincinnati, Cincinnati, OH

Abstract: mTOR pathway mutations are associated with autism, cognitive dysfunction and epilepsy. Humans with mTOR pathway mutations often present with mosaic disruptions of gene function, producing lesions that range from focal cortical dysplasia to hemimegalencephaly. In animal models, loss of the mTOR pathway negative regulator PTEN from hippocampal dentate granule cells leads to neuronal hypertrophy, increased dendritic branching and aberrant basal dendrite formation. These mice exhibit dentate hyperexcitability and spontaneous seizures. Here, we tested the hypothesis that the number of PTEN knockout (KO) cells regulates the severity of the epilepsy phenotype. Utilizing a conditional KO approach, PTEN was selectively deleted from dentate granule progenitor cells, and subsequent progeny, at 2-3 weeks post-natal; the deletion yielded mice with 2-22% of granule cells lacking PTEN. Control (n=13) and PTEN KO mice (n=22) between 8-12 weeks old were implanted with either cortical ECoG electrodes or a hippocampal depth electrode for 24/7 video-seizure monitoring. Additional PTEN mice were bred to contain a Cre-induced Archaerhodopsin (Arch) construct, and PTEN gene deletion was initiated to yield mice with 4-23% KO cells. Acute hippocampal slices were prepared from adult (9-23 weeks old) control Arch-expressing (n=5), Arch-PTEN KO (n=17), and PTEN KO (no Arch, n=6) mice. Evoked responses were recorded from the granule cell layer during perforant path stimulation in baseline conditions, during inhibition of Arch expressing cells, and during a recovery phase. Increasing the number of KO cells produced a transition from epileptiform activity and focal hippocampal seizures to cortical seizures *in vivo* and a progressive increase in hippocampal circuit pathophysiology in acute slices. Moreover, selectively silencing KO cells was sufficient to restore normal network behavior in mice with low KO cell loads, while mice with higher KO loads had a decrease in abnormal circuit behavior. These findings demonstrate that the accumulation of abnormal, KO granule cells can mediate the transition from a pre-clinical to clinical epileptic state. Future studies aim to delineate the impact of KO cells on surrounding neurons that may further contribute to the severity of the epileptic phenotype.

Disclosures: C.L. LaSarge: None. **R.Y. Pun:** None. **S.C. Danzer:** None.

Poster

204. Astrocyte Biology: Cellular, Molecular, and Genetic Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 204.01/DP04/B93

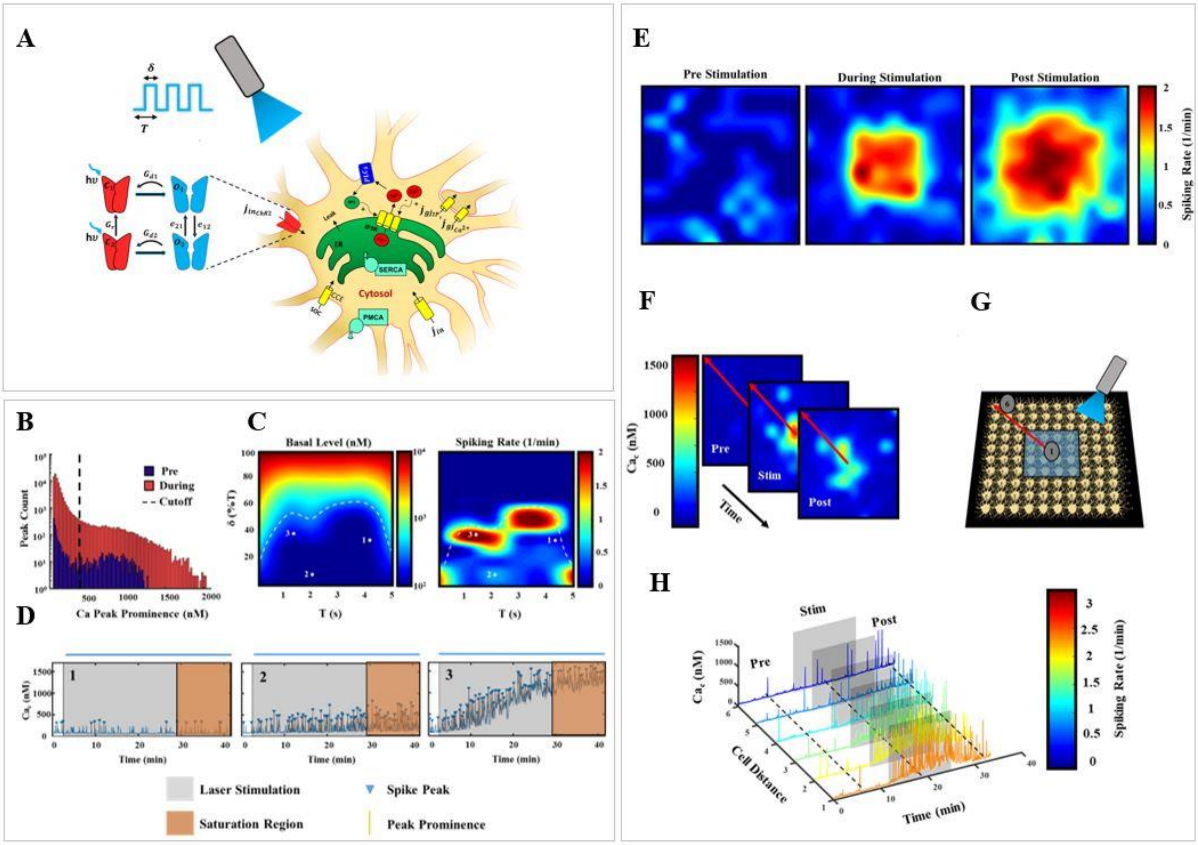
ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: B.11. Glial Mechanisms

Support: BME Coullter SEED grant, Department of Biomedical Engineering, FIU

Title: Unraveling ChR2 driven calcium dynamics in astrocytes - A call for new interventional paradigms

Authors: *L. BALACHANDAR[1], A. MOSHKFOROUSH[1], C. MONCION[1], K. A MONTEJO[3], M. PEREZ[2], E. CASTANO[2], J. RIERA[1];
[1]Biomed. Engin., [2]Biol. Sci., Florida Intl. Univ., Miami, FL; [3]Biomed. Engin. and Physiology, Neurosurg., Mayo Clin., Rochester, MN

Abstract: Control of astrocytes via modulation of Ca^{2+} oscillations using techniques like optogenetics can prove to be crucial in therapeutic intervention of a variety of neurological disorders. However, a systematic study quantifying the effect of optogenetic stimulation in astrocytes is yet to be performed. Here, we propose a novel stochastic Ca^{2+}dynamics model that incorporates the light sensitive component - Channelrhodopsin 2 (ChR2) (Fig. 1A) and verify it using an *in situ* approach. Using the model, we studied the effect of various pulsed light stimulation paradigms on astrocytes for select variants of ChR2 (wild type, ChETA and ChRET/TC) in both an individual and a network of cells. We employed a knock-in murine model expressing ChR2 in astrocytes and used live brain slices for validating our computational model. Our results exhibited a consistent pattern of Ca^{2+} activity among individual cells in response to optogenetic stimulation, i.e., showing steady state regimes with increased Ca^{2+} basal level and spiking probability (Fig.1 B-D). Results from our global sensitivity analysis indicated that directing variants towards the first open state of the photo-cycle of ChR2 (O_1) enhances spiking activity in astrocytes during optical stimulation. We also observed propagation of the Ca^{2+} spiking probability resulting in a network-wide effect on Ca^{2+} dynamics (Fig 1 E-H). Evaluation of the effect of astrocytic ChR2 expression (heterogeneity) on Ca^{2+} signaling revealed that the optimal stimulation paradigm of a network does not necessarily coincide with that of an individual cell. Simulation for ChR2 incorporated astrocytes suggest that maximal activity of a single cell reduced the spiking probability of the network of astrocytes at higher degrees of transduction efficiency due to elevation of basal Ca^{2+} beyond physiological levels. Collectively, the framework presented in our study provides valuable information in the selection of paradigms that elicit optimal astrocytic activity using existing ChR2 constructs, as well as aid in the engineering of future optogenetic constructs.



Disclosures: **L. Balachandar:** None. **A. Moshkforoush:** None. **C. Moncion:** None. **K. A Montejo:** None. **M. Perez:** None. **E. Castano:** None. **J. Riera:** None.

Poster

204. Astrocyte Biology: Cellular, Molecular, and Genetic Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 204.02/B94

Topic: B.11. Glial Mechanisms

Title: Developmental exposure to bisphenol A induces astrocytes activation in rat hippocampus

Authors: ***S. L. NORI**[1], P. DI PIETRO[2], V. NICOLIN[3], R. D'AURIA[2], R. MECCARIELLO[4], C. VECCHIONE[2], A. VIGGIANO[2], A. SANTORO[2];
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Abstract: Bisphenol A (BPA) is a synthetic xenoestrogen diffused worldwide. It leaches out from tin cans and polycarbonate plastic containers and, getting into food and drinks, it accumulates in tissues even in normal conditions of use, like washing and sterilization. As a result, humans are chronically exposed to BPA which can pose a potential health risk. Several studies proposed that BPA can affect synaptic plasticity, inhibit neurogenesis, and induce neuronal autophagy and apoptosis, however very few data are reported about the morphological modification of neuroglia, and data about the effects of BPA on astrocytes are still scarce and inconclusive. Indeed, neuroglia plays a crucial role in supporting neuronal functions including signal conduction and synaptic pruning, therefore perturbing this niche could alter neuronal differentiation and functions potentially leading to neurological disorders and cognitive impairments. To investigate whether BPA could induce astrocytosis, we evaluated glia number and morphology in the hippocampus of 5 female rats from pregnant females who begun to receive BPA (0.1 mg/L in drinking water) or vehicle (control group) after the coupling period and during all over lactation and weaning. Experimental protocols were approved by the local Ethical Committee. At 17PND, all rats were sacrificed and brains removed and processed to perform immunohistochemistry of the hippocampus, a brain region known to be involved in memory and learning. Immunofluorescence analysis showed that BPA enhanced Glial Fibrillary Acidic Protein (GFAP)- immunoreactive astrocytes and increased astrocytes number exhibiting a typical activated morphology within the dentate gyrus compared to the control group. To assess whether these effects were associated to an increased pro-oxidant state, nitrotyrosine immunoreactivity was determined. Results demonstrated a significant induction of nitrotyrosine positive astrocytes, suggesting that BPA could cause neurotoxic effects by inducing astrocytes activation in hippocampus. Taken together these data support that BPA exposure might contribute to the ethiopathogenesis and progression of neurological disorders and neurodegeneration.

Disclosures: **S.L. Nori:** None. **P. Di pietro:** None. **V. Nicolin:** None. **R. D'auria:** None. **R. Meccariello:** None. **C. Vecchione:** None. **A. Viggiano:** None. **A. Santoro:** None.

Poster

204. Astrocyte Biology: Cellular, Molecular, and Genetic Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 204.03/B95

Topic: B.11. Glial Mechanisms

Support: NSERC 101850
Carleton University, CORIS 106815
CFI
Canada Research Chairs
CIHR

Title: A new role for cortical astroglial cells in sexual differentiation of the cerebral cortex

Authors: *G. M. RURAK[1], S. SIMARD[1], A. VAN GEEL[1], J. STEAD[1], B. WOODSIDE[2,1], G. COPPOLA[3], N. SALMASO[1,3];
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Abstract: The study of sexual differentiation of the cerebral cortex has largely focused on neurons. However, astroglial cells play functional roles at all stages of cortical development; including forming synapses and neural networks. Although astroglial cells express estrogen, progesterone and androgen receptors, their role in cortical sexual differentiation has largely been ignored. In this study, we characterised cortical astroglial cells in male and female mice from postnatal day (P) one to adulthood. To achieve this, we employed a multi-model approach. Using a transgenic mouse line expressing GFP bound to a ribosomal protein under the pan-astroglial promoter Aldh1l1, we quantified the total number of astroglial cells, the expression of astroglial-associated cytoskeletal proteins, and morphological subtypes. We also used translating ribosome affinity purification in conjunction with RNASeq to phenotype the translatome of cortical astroglial cells at P1, P4, P7, P14, P35 and in adults. We found 1) significant developmental changes with respect to the number and phenotype of astroglial cells and 2) sex differences in the number of astroglia showing stem cell potential both in the early postnatal period and in adulthood. Surprisingly, whole astroglial translatome analysis showed few basal sex differences in cortical astroglia across all stages of development, however we observed a robust sex difference in the astroglial-specific translatome at P1. Interestingly, many of the differentially expressed genes were associated with synapse and network formation. Because P1 is critical for sexual differentiation of the male brain, we hypothesized that direct stimulation of astroglia by estrogen might induce sex-specific cortical network development; we validated this hypothesis by using an aromatase inhibitor in neonatal males to block sexual differentiation. Together, our data suggest a potential new role for astroglial cells as key regulators of cortical sexual differentiation.

Disclosures: G.M. Rurak: None. **A. Van Geel:** None. **B. Woodside:** None. **G. Coppola:** None. **N. Salmaso:** None.

Poster

204. Astrocyte Biology: Cellular, Molecular, and Genetic Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 204.04/B96

Topic: B.11. Glial Mechanisms

Support: BMBF JenAge 0315581
BMBF Irestra 16SV7209

DFG FOR 1738
TMWWDG RegenerAging-FSU-I-03/14

Title: Off-target recombination labels microglia in GLAST-CreERT2 mice

Authors: S. AFZAL, A. URBACH, K. KIRMSE, C. FRAHM, O. W. WITTE, *S. SCHMIDT;
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Abstract: GLAST-CreERT2 mice are widely used as driver line for cell-type specific genome manipulations in astrocytes. Here we report off-target recombination in the GLAST-CreERT2 mice line in cortical microglia. In double heterozygous male GLAST-CreERT2:Ai14 mice, aged 60-90 days, Tamoxifen (Tx) was administered at different dosages (0, 45, 180 mg/kg bw) by single oral gavage to express the red fluorescent cre-reporting protein tandem dimer Tomato. Five days after Tx-application, immunohistochemistry was performed on coronal brain sections. Co-localization analysis revealed that many tdTomato+ cells were negative for the astrocytic marker S100b. The majority of these tdTomato+/S100b- cells were found to be immunopositive for Iba1 suggesting that they are microglial in nature. After low-dose Tx-application with 45 mg/kg bw, the recombination rate was even higher in Iba1+ as compared to S100b+ cells. In addition, morphological characteristics of tdTomato+/Iba1+ cells matched those reported for resting microglia. We conclude that GLAST-CreERT2 mice should be used with caution if astrocyte-specific Cre expression is required.

Disclosures: S. Afzal: None. **A. Urbach:** None. **K. Kirmse:** None. **C. Frahm:** None. **O.W. Witte:** None. **S. Schmidt:** None.

Poster

204. Astrocyte Biology: Cellular, Molecular, and Genetic Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 204.05/B97

Topic: B.11. Glial Mechanisms

Support: Applebaum fellowship support

Title: Morphology influence the astrocytic bioenergetic preferences but not the chemokine release *in vitro*

Authors: *P. KABIRAJ, C. MCCARTHY, O. GAKH, R. JOHNSON, C. LUCCHINETTI, C. L. HOWE;
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Abstract: Change in morphology, shape and size has been linked to the reactive state of astrocytes. Whether a morphological change of astrocytes is regulated by the bioenergetic

preferences similar to immune cells is under speculation. Several studies have linked neurodegenerative diseases like AD, ALS, HD, MS, hepatic encephalopathy, epilepsy to astrocytic metabolic abnormality as well as the reactive state of astrocytes. Thus far no studies been directed to investigate the different states of astrocytes in relation to the bioenergy preferences. This study is focused on unraveling the bioenergetic preferences of hypertrophic and normal astrocytes. Astrocytes were cultured in FBS or HbEGF to mimic the hypertrophic or normal astrocytes respectively. When grown in HbEGF-media condition, astrocytes are bio-energetically more quiescent than FBS-media grown astrocytes. We also identified that morphological change due to FBS makes astrocytes more glycolytic but do not alter the Lcn2 and CCL5 release after 24h. Astrocytes grown in FBS containing media have higher autophagy flux than those grown in HbEGF containing media and the chemical inhibition of autophagy flux block the FBS induced morphological change. This study also confirmed that FBS grown astrocytes due to its higher energetic state can camouflage the morphological and energetic response to insulin. Our observations concluded that recognition of the bioenergetic quiescence is essential to study astrocyte activity.

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Poster

204. Astrocyte Biology: Cellular, Molecular, and Genetic Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 204.06/B98

Topic: B.11. Glial Mechanisms

Support: AFOSR ASTRONIR FA9550-17-1-0502
AFOSR 3DNeuroglia
AFOSR MURI FA9550-16-1-0052

Title: Impact of actin waves on ion and water membrane dynamics in astrocytes

Authors: *K. M. O'NEILL[1], E. SARACINO[2], L. MAIOLO[3], M. G. MOLA[5], A. CONVERTINO[3], V. GUARINO[4], T. POSATI[2], M. CAPRINI[6], G. FORTUNATO[3], R. ZAMBONI[2], L. AMBROSIO[4], G. P. NICCHIA[5], W. LOSERT[7], V. BENFENATI[2];
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Abstract: It is well known that astrocytes have a crucial role in maintaining homeostasis in the human brain. Notably, a molecular and functional interplay between ion channels, water channel aquaporin 4 (AQP4), and calcium signalling has been established and is mainly localized to the endfeet of astrocytes. Moreover, the role of actin remodelling is essential to these homeostatic processes. However, many studies have relied on imaging fixed samples of astrocytes, which have static actin. Conclusions have also been made based on work where primary astrocytes display a flat, polygonal shape and do not have the morphological, structural, or functional features of *in vivo* astrocytes. To address this limitation, our previous work has validated nanostructured surfaces, such as silicon-nanowires (Au/SiNw), polycaprolactone (PCL) electrospun nanofibers, and hydrotalcite nanoparticles films (HTlc), as novel glial interfaces that enable *in vitro* astrocytes to grow and differentiate into an *in vivo*-like state by responding to 2D and 3D substrate topography. In the present work, we seek to study actin dynamics in primary cultures of neocortical rodent astrocytes. To this end, we cultured astrocytes on poly-D-lysine coated glass, as is standard in the field, and on the aforementioned nanostructured surfaces to understand astrocytic responses to the mechanical environment and to gain more reliable results on *in vivo*-like astrocytic processes. Actin visualization was accomplished by transduction with actin-GFP at 48 h prior to timelapse confocal imaging. During imaging, changes in actin dynamics were analyzed in response to a "triggering" solution, such as high potassium extracellular medium or hypotonic solutions, compared to a standard external medium with the purpose of understanding how the cytoskeleton responds to changes in the ionic environment. We successfully captured actin dynamics in astrocytes at 3 and 7 days *in vitro* and observed dynamic cytoskeletal changes in response to alterations in both the mechanical and ionic extracellular environments. Our work also underscores the importance of AQP4 in regulating astrocytic responses. Our results reveal a new understanding of the role of actin in the mechanisms underpinning astrocyte homeostatic function at multiple length scales. We also provide insight into the ability of the actin cytoskeleton in astrocytes to change dynamically in response to different substrate topography, which has not before been studied.
* O'Neill & Saracino contributed equally to this work.

Disclosures: K.M. O'Neill: None. **E. Saracino:** None. **L. Maiolo:** None. **M.G. Mola:** None. **A. Convertino:** None. **V. Guarino:** None. **T. Posati:** None. **M. Caprini:** None. **G. Fortunato:** None. **R. Zamboni:** None. **L. Ambrosio:** None. **G.P. Nicchia:** None. **W. Losert:** None. **V. Benfenati:** None.

Poster

204. Astrocyte Biology: Cellular, Molecular, and Genetic Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 204.07/B99

Topic: B.11. Glial Mechanisms

Support: Minnesota Partnership for Biotechnology and Medical Genomics
Mayo Foundation

Title: The effect of electrical stimulation at different frequencies on astrocyte intracellular vesicle mobility

Authors: *Y. WANG[1], T. P. BURGHARDT[2], G. A. WORRELL[1], H.-L. WANG[1];
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Abstract: Extracellular vesicles (EVs) are released by all cells and are fundamental for intercellular transport, cell-to-cell communications and many other biological functions. EVs carry cargos such as proteins, nucleic acids, lipids and metabolites. Delivery of vesicles to the plasma membrane in the cell is controlled by intracellular vesicle mobility. We have previously shown that EVs release and MicroRNA and protein cargo can be modulated with electrical stimulation, but, the cellular mechanism is unknown. In this work, we study the effect of electrical stimulation on the mobility of intracellular vesicles in cultured astrocytes. We labeled intracellular vesicles with GFP-attached membrane protein (VAMP3 or CD63) and monitored their intracellular trafficking in cultured rat astrocytes with and without externally applied electrical stimulation at different frequencies. We found that vesicle mobility for both direct and indirect movement increased more than 20% over control with 2 Hz electrical stimulation, but remained unchanged with higher frequencies (20 Hz and 200Hz) electrical stimulation. This study raises an interesting question about the effect of low frequency external electric fields on astrocyte derived EVs and may be important for the reported impact of slow-wave sleep on EVs release.

Disclosures: Y. Wang: None. **T.P. Burghardt:** None. **G.A. Worrell:** None. **H. Wang:** None.

Poster

204. Astrocyte Biology: Cellular, Molecular, and Genetic Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 204.08/B100

Topic: B.11. Glial Mechanisms

Support: CONACyT-Mexico fellowship 487731

Title: Dynamic DNA methylation as a mechanism of epigenetic regulation in glial cells under excitotoxicity

Authors: *A. G. RODRIGUEZ-CAMPUZANO, A. ORTEGA;
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Abstract: The constant exposure to several xenobiotics has a significant impact in the central nervous system (CNS), resulting in an excitotoxic process induced by a massive release of the main excitatory neurotransmitter L-glutamate (Glu). Overstimulation of post-synaptic glutamate receptors leads to a disturbance of intracellular calcium homeostasis that is linked to neuronal death. Hence, the extracellular levels of Glu are tightly regulated mainly through glial Glu transporters (EAATs) (EAAT1, EAAT2). The regulation of the expression of these transporters under excitotoxic concentrations of Glu has been widely studied, however, the results on the regulation of EAAT1 expression in the long term are inconclusive. Thus far, it has been observed that Glu regulates its own removal from the synaptic cleft through a process mediated by Ca^{2+} -permeable AMPA receptors, PKC and the transcription factor YY1. Taking into consideration that YY1 as a member of the Polycomb group can be part of repressive and activating chromatin remodeling groups, it can indirectly target DNA methyltransferases (DNMTs) or dioxygenases of methylated cytosines such as TET, to their target sequences. Being the EAAT1 promoter an important target of YY1 in the CNS and that its regulation in response to excitotoxic stimuli is associated with the development and progress of neurodegenerative disorders, herein we have used MIO-M1 cells as a human glial model to address the epigenetic regulation of EAAT1. We have been able to detect changes in mRNA and protein levels of YY1, DNMT1, DNMT3B and EAAT1 in response to excitotoxic levels of Glu at 24, 48 and 72 h. Concomitantly, parallel global methylation patterns change under the same conditions. Moreover, in order to gain insight about a plausible involvement of DNA methylation in EAAT1 uptake function, we evaluated the activity of this transporter after treatment with 5-Aza-2'-deoxycytidine. These results strongly suggest that dynamic DNA methylation of EAAT1 promoter is involved in the epigenetic regulation of Glu turnover and by these means in glia-dependent synaptic transmission modulation.
Keywords: GLIA, GLAST / EAAT1, YY1, PKC, DNMTs

Disclosures: A.G. Rodriguez-campuzano: None. **A. Ortega:** None.

Poster

204. Astrocyte Biology: Cellular, Molecular, and Genetic Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 204.09/B101

Topic: B.11. Glial Mechanisms

Support: NIH grant NS057499
NIH T32 grant AG020506

Title: The role of astrocyte calcium signals in production of and response to inflammatory cytokines

Authors: *M. NOVAKOVIC[1], M. PRAKRIYA[2];
[1]Pharmacol., Northwestern Univ., Chicago, IL; [2]Northwestern Univ. - Chicago, Chicago, IL

Abstract: Astrocytes play diverse roles in the nervous system such as neurotransmission and metabolic support, and growing evidence indicates that they mediate inflammation by responding to and releasing cytokines. These cytokines, such as TNF-α and IL-6, are elevated in a growing list of pathologies including Alzheimer's Disease and other dementias. In immune cells, cytokines are produced in response to intracellular Ca^{2+} elevations from activation of Store-Operated Ca^{2+} Entry (SOCE), but whether these mechanisms regulate astrocytic cytokine production remains unclear. We have recently shown that SOCE mediated by a Ca^{2+} channel, Orai1, and ER sensor, Stim1, is a major mechanism for mobilizing calcium in astrocytes. Therefore, sought to understand the role of SOCE in cytokine production in astrocytes. We found that in cultured hippocampal astrocytes, stimulation of SOCE with GPCR agonists induced the induction of multiple cytokines including IL-6, IL-4, and MIP1α. This induction was lost in cells with genetic deletion of Orai1. SOCE induction also activated transcription factors NFAT and NFκB in an Orai1-dependent manner. Consistent with involvement of these transcription factors in the Ca^{2+}-dependent regulation of cytokines, induction of IL-6 was abolished by inhibition of NFAT but not NFkB, indicating that Orai1 activation of NFAT is a critical aspect of IL-6 production. In addition to the increasingly recognized role of astrocytes in releasing cytokines, previous work has established that astrocyte physiology and function is altered by microglial factors, including C1q, TNF-α, and IL-1α. Given the host of cellular functions modulated by Ca^{2+}, these broad changes in astrocyte physiology raise the possibility that cytokines evoke autocrine or paracrine effects on SOCE. Consistent with this idea, TNF-α was found to reduce SOCE in astrocytes. TNF-α also increased the expression of the inhibitory pore subunit, Orai2, suggesting that SOCE inhibiton by TNF-α is accomplished by an altered Orai2:Orai1 subunit ratio. Consistent with this, TNF-α-mediated SOCE inhibition was lost in Orai2 KO mice. These results reveal a novel mechanism of reciprocal regulation of calcium signaling by cytokines and highlight the critical role of SOCE for cytokine production by astrocytes.

Disclosures: M. Novakovic: None. **M. Prakriya:** None.

Poster

204. Astrocyte Biology: Cellular, Molecular, and Genetic Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 204.10/B102

Topic: B.11. Glial Mechanisms

Support: NTU Nanyang Assistant Professorship startup grant

Title: Transcriptional regulation of glycogen metabolism in astrocytes

Authors: W. L. LIM[1], A. W. J. TAN[1], *T. H. CH'NG[2];
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Abstract: Brain glycogen is predominantly stored in astrocytes and can be rapidly broken down into lactate which is required for the formation of long term memory. Glycogen metabolism including breakdown and synthesis are thus critical processes during memory formation. Nevertheless, the transcriptional regulation of glycogen synthesis in astrocytes including the transcription factors involved in the process still remain relatively undefined. While CREB has been identified as a key transcription factor associated with neuronal activity-driven transcription in astrocytes, the role of CREB in glycogen metabolism in astrocytes is not defined. Here, we present data for the involvement of CREB1 in regulation gene expression during glycogen metabolism. Stimulation of astrocytes with vasoactive intestinal peptide (VIP) lead to glycogen breakdown and synthesis that is dependent on calcium signaling and Protein Kinase C (PKC) activation, leading to phosphorylation of CREB at PKC associated serine residues (S133 and S121). We show evidence that expression of genes associated with glucose intake and glycogen metabolism including *ptg*, *cebpβ* and *slc2a1* are selectively regulated by PKC-driven CREB activation during VIP stimulation. Finally, we examine the role of intracellular calcium and CRTC family of transcriptional coactivator during glycogen metabolism.

Disclosures: W.L. Lim: None. **A.W.J. Tan:** None. **T.H. Ch'ng:** None.

Poster

204. Astrocyte Biology: Cellular, Molecular, and Genetic Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 204.11/C1

Topic: B.11. Glial Mechanisms

Support: Woodruff School of Mechanical Engineering Startup Fund
Petit Institute Seed Grant
NIH ES025661

Title: Exogenous heme dysregulates Akt/mTOR signaling and downstream immune function in astrocytes

Authors: *S. SANKAR[1], K. SHAH[1], D. HANNA[2], M. BRYANT[1], A. REDDI[2], L. WOOD[3];
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Abstract: Elevated levels of the blood-derived factor, heme, drive pathology in a number of acute brain injuries and chronic neurodegenerative conditions, including ischemic stroke, traumatic brain injury, and Alzheimer's disease (AD). While known for its oxygen-carrying

properties, heme also has immunomodulatory functions and plays a prominent role as a modulator of cell signaling cascades, such as the mitogen activated protein kinase pathway, in the peripheral immune system. However, the role of heme signaling in modulating the neuro-immune system remains largely uncharacterized. Prior work has shown that heme suppresses critical astrocyte immune functions, such as scavenger activity and cytokine expression. Given that astrocyte immune dysfunction is a prominent feature of neuropathological conditions such as AD and that the Akt/mTOR phospho-signaling pathway regulates a number of immune functions in astrocytes, we sought to elucidate the effects of exogenous heme on Akt/mTOR signaling and downstream immune phenotypes in primary murine astrocytes *in vitro*. Using a Luminex multiplexed immunoassay and discriminant partial least squares regression analysis, we found that exogenous heme rapidly upregulates phosphorylation of a profile of phospho-proteins in the Akt/mTOR pathway, including Akt, mTOR, and IGF1R. Using a novel, genetically encoded, fluorescent heme sensor to monitor astrocyte heme uptake together with quantifying Akt and mTOR signaling dynamics over the course of 24 hours, we found that exogenous heme significantly increases intracellular labile heme pools concomitant with peak upregulation of Akt and mTOR phosphorylation. Furthermore, we found that blocking cell surface receptor tyrosine kinases (ie. IGF1R, VEGFR) partially attenuates Akt/mTOR upregulation in response to exogenous heme. Together, these data suggest that heme may dysregulate phospho-protein signaling through both intra- and extra- cellular mechanisms. Finally, we found that heme significantly upregulates the anti-inflammatory protein heme oxygenase-1 (HO-1) in astrocytes, and that HO-1 expression is suppressed upon inhibition of mTOR with rapamycin. This work identifies novel signaling functions of heme in astrocytes, which could contribute to the development of heme-based therapeutics for neurodegenerative conditions.

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Poster

204. Astrocyte Biology: Cellular, Molecular, and Genetic Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 204.12/C2

Topic: B.11. Glial Mechanisms

Support: US Army NETRP Grant No. W81XWH-04-1-0444

Title: Lactate administration recapitulates the astrocyte-specific neuroplastic effects of exercise

Authors: *A. J. LUNDQUIST, T. J. GALLAGHER, G. M. PETZINGER, M. W. JAKOWEC; Neurol., USC, Los Angeles, CA

Abstract: Aerobic exercise is a form of neuroplasticity that is capable of modifying the progression of motor-related neurodegenerative diseases such as Parkinson's disease (PD). However, the molecular mechanisms that underlie this consequence of exercise are unknown. Our previous studies have shown that aerobic exercise changes cerebral blood flow and cellular metabolism in a brain region- and circuit-specific manner. Therefore, we are interested in exploring how peripheral metabolism in exercise may impact and influence such changes to promote neuroplasticity. Lactate, a molecule excreted by muscles and consumed by the brain during strenuous exercise, is an important energetic molecule that is key to the metabolic and plasticity connection between astrocytes and neurons. Lactate also functions as a signaling molecule through its G_i-protein coupled receptor HCAR1 (hydroxycarboxylic acid receptor 1). Due to the connection between lactate and plasticity as well as its production during exercise, lactate represents a potential molecular mechanism for the exercise-induced neuroplasticity that benefits PD patients. This project sought to uncover the role of lactate in the neuroplastic effects of exercise. *In vivo*, the administration of L-lactate caused an increase in the transcription of metabolic and plasticity related genes in the striatum that are also upregulated as a consequence of aerobic exercise; however, evidence of *de novo* synaptogenesis was not found, suggesting that lactate effects are astrocyte-specific. Additionally, application of either L-lactate or 3,5-DHBA (3,5-dihydroxybenzoic acid, an agonist of HCAR1) to primary astrocytes mimicked L-lactate effects, demonstrating a likely G-protein coupled receptor pathway through which L-lactate creates its effects. Finally, the impact of lactate on astrocyte metabolism was measured through lactate excretion, metabolic flux, and mitochondrial biogenesis following the application of L-lactate, D-lactate, and 3,5-DHBA. Together, the data collected demonstrate that exogenous lactate, only in its biologically relevant form (L-lactate), causes metabolic and transcriptional changes to astrocytes that support increased plasticity. The stimulus that triggers these changes appears to be activation of the lactate receptor, HCAR1. These results suggest that lactate is likely an important molecular component of exercise-induced neuroplasticity and present possible therapeutic targets for modification of disease progression in patients with PD.

Disclosures: A.J. Lundquist: None. **T.J. Gallagher:** None. **G.M. Petzinger:** None. **M.W. Jakowec:** None.

Poster

204. Astrocyte Biology: Cellular, Molecular, and Genetic Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 204.13/C3

Topic: B.11. Glial Mechanisms

Support: KIST Institutional Grant
Creative Research Initiative Program

Title: AAV mediated astrocyte specific gene expression under human ALDH1L1 promoter

Authors: *S. LEE[1], C. J. LEE[2];
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Abstract: Adeno-associated virus (AAV)-mediated gene delivery has been proposed to be an essential tool of gene therapy for various brain diseases. Among several cell types in the brain, astrocyte has become a promising therapeutic target for brain diseases, as more and more contribution of astrocytes in pathophysiology has been revealed. Until now, genetically targeting astrocytes has been possible by utilizing the glial fibrillary acidic protein (GFAP) promoter. In some brain areas including thalamus, however, the GFAP expression in astrocytes is reported to be low, making it difficult to genetically target astrocytes using GFAP promoter. To study the function of astrocytes in thalamus, which serves as a relay station, there is a great need for identifying an alternative astrocyte-specific promoter in thalamus. Recently, a new astrocyte-specific promoter of ALDH1L1 has been identified. However, it has not been examined in thalamus. Here we developed and characterized an AAV vector expressing Cre recombinase under the human ALDH1L1 promoter, AAV-hALDH1L1-Cre. To test the cell-type specific expression of AAV-hALDH1L1-Cre, AAV virus was injected into several brain regions of Ai14 (RCL-tdTomato) mouse, which reports Cre activity by tdTomato expression. In thalamus, we observed that tdTomato was found mostly in astrocytes (91.71%), with minimal occurrence in neurons (2.67%). In contrast, tdTomato signal was observed in both neurons and astrocytes of the amygdala (neuron: 68.13%, astrocyte: 28.35%) and hippocampus (neuron: 76.25%, astrocyte: 18.00%), which is consistent with the previous report showing neuronal gene expression under rat ALDH1L1 promoter. Unexpectedly, tdTomato was found mostly in neurons (91.98%) with minimal occurrence in astrocytes (6.66%) of the medial prefrontal cortex. In conclusion, hALDH1L1 promoter shows astrocyte-specificity in thalamus and may prove to be useful for targeting thalamic astrocytes in mouse.

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

Poster

204. Astrocyte Biology: Cellular, Molecular, and Genetic Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 204.14/C4

Topic: B.11. Glial Mechanisms

Support: Title V R25GM110513
RISE P031S130068

Title: The involvement of polyamine uptake and synthesis pathways in proliferation of neonatal astrocytes

Authors: *C. J. MALPICA-NIEVES[1], D. E. RIVERA-APONTE[1], F. TEJEDA-BAYRON[1], D. RÍOS-OTERO[2], A. ZAYAS-SANTIAGO[3], O. PHANSTIEL[5], M. J. EATON[1], S. SKATCHKOV[4];
[1]Biochem., [2]Physiol., [3]Pathology and Lab. Med., [4]Biochem. and Physiol., Univ. Central Del Caribe-School of Med., Bayamon, PR; [5]Dept. of Med. Educ., Univ. of Central Florida, Orlando, FL

Abstract: Polyamines (PAs) are essential to promote cell growth, survival, and proliferation. In the adult brain, where cell differentiation and tissue growth is mainly completed, PAs including spermidine and spermine are predominantly accumulated in glial cells. The biosynthetic enzyme, ornithine decarboxylase (ODC), which synthesizes the spermidine precursor putrescine is found in neurons. This indicates that adult glial cells accumulate PAs not by synthesis, but by uptake. In contrast, during the early postnatal period, the situation is different. In rat retina, we found that PAs and their biosynthetic enzymes are expressed in both glia and neurons during this neonatal period, whereas the biosynthetic machinery was found only in a few retinal neurons in adult. The purpose of the present study was to determine if uptake or synthesis of PAs supports cell proliferation in neonatal brain. With the use of α-difluoro-methylornithine (DFMO) an inhibitor of ODC and a trimer44NMe polyamine transport inhibitor (PTI), we depleted the endogenous synthesis and cellular uptake of PAs in primary cultured cortical astrocytes from Sprague Dawley rats (1-3 day old). Using a Live/Dead Cell Assay, we determined the working concentrations of DFMO and PTI in astrocytes. We then measured cell proliferation at 1, 2 and 7 days using a TC20 cell counter. Our results demonstrate that combined treatment with DFMO (5 mM) + PTI (30 µM) or DFMO alone reduced astrocyte proliferation by 50% during 7 days of treatment. However, when PTI was used alone, proliferation was comparable to control suggesting that endogenous synthesis in neonatal astrocytes was present which we confirmed by immunoblotting for ODC. When endogenous synthesis was blocked (in the presence of DFMO), we added spermidine (10 µM) to the culture media to replenish the cells with exogenous PAs and this treatment rescued cell proliferation by 46%. When both synthesis and uptake of PAs were inhibited (DFMO + PTI), exogenous spermidine (10 µM) was no longer able to support cell proliferation. In conclusion, our data indicate that astrocytes cultured from neonatal rats synthesize sufficient quantities of PAs de novo to support cell proliferation, but may also take up exogenous PAs to support proliferation when synthesis is inhibited.

Disclosures: C.J. Malpica-Nieves: None. **D.E. Rivera-Aponte:** None. **F. Tejeda-Bayron:** None. **D. Ríos-Otero:** None. **A. Zayas-Santiago:** None. **O. Phanstiel:** None. **M.J. Eaton:** None. **S. Skatchkov:** None.

Poster

204. Astrocyte Biology: Cellular, Molecular, and Genetic Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 204.15/C5

Topic: B.11. Glial Mechanisms

Support: NSERC 101850

Title: Age and sex-dependent effects of environmental enrichment on behaviour and astroglial plasticity

Authors: *K. CHANDLER[1], G. COPPOLA[2], H. DOSSO[1], S. SIMARD[1], N. SALMASO[1]; [1]Neurosci., Carleton Univ., Ottawa, ON, Canada; [2]Child Study Ctr., Yale Univ., New Haven, CT

Abstract: Environmental enrichment (Enr) has been shown to increase cognitive abilities such as problem solving, place learning, and memory in rodents. Previous work has demonstrated that 2 weeks of environmental enrichment is sufficient to increase cognitive abilities in the Morris Water Maze and increase the GFAP+ stem cell pool in juvenile mice. However, in adults, longer-term Enr protocols of six weeks or more are typically used to induce behavioural and functional recovery; few studies have examined the effects of short-term Enr. We hypothesized that short-term Enr would not be sufficient to induce changes in cognitive behavior and to increase the stem cell pool/neurogenesis in adults. To test this we exposed juvenile and adult mice to two weeks of Enr and 1) tested changes in cognitive and anxiety-like behaviour and 2) used neurosphere assays to examine the proliferative potential of neural stem cells in the subventricular zone and dentate gyrus. To understand the underlying mechanism of environmental enrichment and its effects on changes in astroglial gene expression, we used translating ribosome affinity purification in conjunction with RNASeq to phenotype the translatome of hippocampal astroglial cells in response to Enr in juvenile, young and aged males and females . As hypothesized, we found that short-term Enr augmented learning and memory in juvenile mice, but not in the adult mice. These changes were paralleled by an increase in proliferation of the stem cell pool in juveniles that was less pronounced in adults, together suggesting an age-related decrease in NSC potential and plasticity in response to short-term Enr. Finally, our sequencing data suggests that astroglial cells of male and female mice respond differently to Enr both at baseline and across time-points.

Disclosures: K. Chandler: None. **G. Coppola:** None. **H. Dosso:** None. **S. Simard:** None. **N. Salmaso:** None.

Poster

204. Astrocyte Biology: Cellular, Molecular, and Genetic Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 204.16/C6

Topic: B.11. Glial Mechanisms

Title: Adult zebrafish astroglial response to olfactory organ damage in the olfactory bulb

Authors: *J. SCHEIB, *C. BYRD-JACOBS;
Biol. Sci., Western Michigan Univ., Kalamazoo, MI

Abstract: The brain requires a degree of neuroplasticity to rewire and repair itself after injury. Humans have evolved to have a limited degree of neuroplasticity, which inhibits the ability to recover from brain trauma. Zebrafish, however, are renowned for their neuroplasticity, and their olfactory system is an excellent model for this. Glia are major mediations of neuroplasticity. Astrocytes, a type of glia, maintain homeostasis within the brain and react during brain trauma by astrogliosis, which is characterized by an increase in cell branching, hypertrophy, and proliferation. Astrogliosis, in mammals, can be both neuroprotective and neurotoxic and, if severe enough or repetitive, may cause scar formation that further hinders neuroplasticity. Zebrafish have previously been shown to lack glial scar formation after direct injury. However, it is unknown if damage to the periphery will cause astrogliosis in central structures and, if this is repetitive, if astrocytes will retain their astrogliosis morphology to form a scar. In this study, we repetitively damage the peripheral organ of the zebrafish olfactory system and analyze changes in astroglial morphology and proliferation patterns in the olfactory bulb. Our hypothesis is that mechanical damage to the olfactory organ will cause astrogliosis in the olfactory bulb.
To induce peripheral trauma, a wax plug was inserted into the nasal cavity of adult zebrafish to crush the olfactory organ every 12 hours up to 7 days. At early time points, we found a significant increase in astroglial labeling in the affected bulb when compared to the internal control. Astroglial branches appeared to increase in number and size. Interestingly, this effect was seen only at earlier time points. By the third day of treatment there was no significant difference in astroglial labeling between the affected bulb and the internal control bulb. A significant increase in astroglial proliferation was observed also at 24 hours after the first insult to the olfactory organ. These data lead us to believe that astrogliosis does occur in the presence of peripheral damage, but this process attenuates by later time points. Further exploration of astrocytes in zebrafish, in particular this apparent astrogliosis attenuation, have the potential to elucidate key differences of astrocyte function. These key differences have the potential to be exploited for medical intervention in brain trauma and disease in humans.

Disclosures: J. Scheib: None. **C. Byrd-Jacobs:** None.

Poster

204. Astrocyte Biology: Cellular, Molecular, and Genetic Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 204.17/C7

Topic: B.11. Glial Mechanisms

Title: FGFR1 signaling in hypothalamic tanycytes

Authors: *N. A. ESTEVE[1], D. J. ROGERS[2], N. DUNN[2], R. TRAN[2], K. M. SMITH[2];
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Abstract: Tanycytes are specialized astroglial cells residing in the hypothalamus, on the border of the third ventricle, and extending ventrally towards the medial eminence. A subset of the tanycyte population has demonstrated neurogenic and gliogenic (particularly astrocytes) properties that could mediate modulation of neural circuitry in neighboring hypothalamic nuclei (i.e. arcuate nuclei). Along with proliferative properties, tanycytes have various functions associated with energy expenditure, appetite, glucose regulation, and hormonal regulation. Understanding tanycyte development and function is important for understanding hypothalamic physiology. Currently, it is believed that tanycytes originate during the perinatal period. During early development, growth factors, such as fibroblast growth factors (FGFs), are highly prevalent and mediate both cellular proliferation and differentiation, in the postnatal period. FGFR signaling appears to be important for postnatal proliferation of astrocytes. Our previous studies demonstrated strong FGFR1 expression in tanycytes of the hypothalamus throughout perinatal and adult time periods. We have generated Nestin-Cre mediated deletion of floxed alleles of Fgfr1. The Nestin promoter directs Cre-mediated recombination to stem cell progenitors throughout the CNS. We have observed that mice lacking Fgfr1 have a reduction in the tanycyte staining surrounding the third ventricle at five months of age. No differences were observed in baseline blood glucose levels, or in the sucrose preference test, between control and FGFR1 mutants. We have further cultured astrocytes isolated from control and Fgfr1 mutant littermates at P2-P4, in order to examine if Fgfr1 influences calcium signaling within these the hypothalamus. These preparations are responsive to glucose and ATP stimulation. We found that Fgfr1 expression influences calcium signaling within hypothalamic tanycytes when stimulated with 25 mM ATP. We observed differences in the number of calcium waves per 30 seconds ($p=.0001$), time between calcium waves ($p=.0051$). Future studies will examine tanycyte number and morphology in the perinatal period. Our studies elucidate the functional roles of Fgfr1 signaling in tanycyte and hypothalamic function.

Disclosures: **N.A. Esteve:** None. **D.J. Rogers:** None. **N. Dunn:** None. **R. Tran:** None. **K.M. Smith:** None.

Poster

204. Astrocyte Biology: Cellular, Molecular, and Genetic Mechanisms

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Topic: B.11. Glial Mechanisms

Support: NJCSCR CSCR13IRG012
NMSS RG-1803-30494

Title: Differential distribution of Hh-responsive astrocytes in the adult mouse CNS suggest region-specific functions

Authors: *M. S. RALLO, H. WANG, Z. XU, Z. XIA, M. P. MATISE;
Neurosci. and Cell Biol., Rutgers Robert Wood Johnson Med. Sch., Piscataway, NJ

Abstract: Sonic hedgehog (Shh) is known to play a critical role in CNS development and in maintaining tissue homeostasis in adults, as well as the response to injury/disease. In the mature mouse CNS, the vast majority of Shh-responsive cells are astrocytes, as revealed by their expression of *Gli1*, a gene that is only activated in cells responding to canonical Hedgehog (Hh) signaling. In the adult brain, Gli1+ cells are located in the deep layers of the cortex, striatum, hippocampus, diencephalon, and brainstem. Notably, many Gli1+ astrocytes make contact with blood vessels, suggesting a potential role in regulating the blood-brain barrier (BBB). However, it remains unclear whether the heterogenous distribution of Gli1+ astrocytes reflects regionally diverse functions.
To address this, we generated a tamoxifen-inducible *Gli1-Cre^{ERT2};Rosa26^{tdTomato}* mouse line to characterize the molecular and functional properties of Gli1+ astrocytes in the adult brain. This line allows isolation of Gli1+ cells by FACS for transcriptome analysis to determine whether there are differences in their regional expression profiles. Histological analysis revealed that the highest concentration of Gli1+ astrocytes in the brain is found around the third ventricle in the hypothalamus, a key region for regulating CNS and systemic homeostasis in mammals. Notably, selective inactivation of Hh signal transduction in astrocytes using a *GFAP-Cre^{ERT2};Smo$^{c/c}$* mouse line resulted in BBB disruption in CNS regions containing Gli1+ astrocytes, most notably in the hypothalamic paraventricular nucleus (PVN), a structure with high blood vessel density. Together, these findings raise the possibility that regulation of BBB permeability by Hh signaling in Gli1+ astrocytes in the hypothalamus plays a unique role in the physiological functions mediated by neurons in this region of the CNS.

Disclosures: **M.S. Rallo:** None. **H. Wang:** None. **Z. Xu:** None. **Z. Xia:** None. **M.P. Matise:** None.

Poster

204. Astrocyte Biology: Cellular, Molecular, and Genetic Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 204.19/C9

Topic: B.11. Glial Mechanisms

Support: NS060677

Title: Assessing astrocyte morphology using Lucifer yellow iontophoresis

Authors: *S. L. MOYE[1], B. DIAZ-CASTRO[1], M. R. GANGWANI[1], B. S. KHAKH[2];
[2]Dept Physiol, [1]Univ. of California Los Angeles Dept. of Physiol., Los Angeles, CA

Abstract: Astrocytes are essential components of neural circuits. They tile the entire central nervous system (CNS) and are involved in a variety of functions which include neurotransmitter clearance, ion regulation, synaptic modulation, metabolic support to neurons, and blood flow regulation. Astrocytes are complex cells that have a soma, several major branches, and numerous fine processes that contact diverse cellular elements within the neuropil. In order to assess the morphology of astrocytes, it is necessary to have a reliable and reproducible method to visualize their structure in detail. We report a simple protocol to perform intracellular iontophoresis of astrocytes using fluorescent Lucifer yellow (LY) dye in lightly fixed brain tissue from adult mice. This method has several features that are useful to characterize astrocyte morphology. First, it allows for three-dimensional reconstruction of individual astrocytes, which is useful to perform morphological analyses on different aspects of their structure. Second, immunohistochemistry together with LY iontophoresis can be utilized to understand the interaction of astrocytes with different components of nervous system. Third, this protocol can be implemented in a variety of mouse models of CNS disorders to rigorously examine astrocyte morphology with light microscopy. LY iontophoresis provides an experimental approach to evaluate astrocyte structure, especially in the context of injury or disease where these cells are proposed to undergo significant morphological changes.

Disclosures: S.L. Moye: None. **B. Diaz-Castro:** None. **M.R. Gangwani:** None. **B.S. Khakh:** None.

Poster

204. Astrocyte Biology: Cellular, Molecular, and Genetic Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 204.20/C10

Topic: B.11. Glial Mechanisms

Support: NIH R01 NS086933-01
LeJeune Foundation
T32AG052371

Title: Immunohistological examination of AKT isoforms in the brain

Authors: *R. MILSTEAD[1], H. WONG[1], J. LEVENGA[3], C. HOEFFER, Jr.[2];
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[3]DSM, Boulder, CO

Abstract: AKT is a central protein kinase involved in a wide variety of cellular signaling pathways, ranging from development to synaptic plasticity maintenance. There are three structurally similar AKT isoforms (AKT1/PKBα, AKT2/PKBβ, AKT3/PKBγ). In mouse models deficient in each individual isoform, certain differences in behavior and physiology are apparent. Additionally, human genetic data suggests a link between certain polymorphisms in the genes for each isoform and increased risk for neurological diseases like schizophrenia and glioblastoma. Recently published data from our group shows that, in the mouse brain, AKT1 and AKT3 are expressed primarily in neurons, and AKT2 is expressed primarily in astrocytes. We have now expanded upon our mouse results using post-mortem human brain tissue. Recapitulating our findings in the mouse brain, we find AKT1-positive staining primarily in neurons and AKT2-positive staining primarily in astrocytes in human brain tissue. Similar expression patterns for AKT isoforms between mouse and human brain tissue supports the use of mouse models for translational work related to AKT function, and understanding the cell-type specific expression of AKT isoforms could lead to better targeted cellular therapies for many psychiatric diseases like schizophrenia and depression and may allow for more specific targeting of AKT in cancers like glioblastoma.

Disclosures: R. Milstead: None. **H. Wong:** None. **J. Levenga:** None. **C. Hoeffer:** None.

Poster

204. Astrocyte Biology: Cellular, Molecular, and Genetic Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 204.21/DP02/C11

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: B.11. Glial Mechanisms

Title: Astrocytic mitochondrial populations in the striatum and hippocampus are morphologically and functionally distinct

Authors: *T. E. HUNTINGTON[1], T. PATTERSON[1], M. VALLE[1], R. SRINIVASAN[2]; [1]Neurosci. and Exptl. Therapeut., Texas A&M Univ., College Station, TX; [2]Dept. of Neurosciecne and Exptl. Therapeudics Texas, Texas A&M Univ. Col. of Med., Bryan, TX

Abstract: Astrocytic Ca^{2+} signals are analogous to neuronal action potentials and have emerged as an important modulator of neural circuits, neurotransmitter clearance, K^+ buffering, and neurovascular coupling. Recent reports show that the majority of astrocytic Ca^{2+} signals occur in mitochondria. However, there remains a gap in our understanding of subcellular and circuit level heterogeneity among mitochondrial Ca^{2+} signals as well as the regulation of astrocytic mitochondrial Ca^{2+} by neurotransmitters *in situ*. To directly monitor Ca^{2+} signals in astrocytic mitochondria in mouse brain slices, we created an AAV construct with a genetically encoded

calcium sensor (GCaMP6f) tagged to the mitochondrial signaling sequence (mito-7) and driven by the astrocyte-specific promoter (GfaABC1D). We segregated astrocytic mitochondrial Ca^{2+} signals into three subpopulations: somatic mitochondria and large or small mitochondria within the territory of the astrocyte. Mitochondrial pools encompassing these three subpopulations displayed significant differences from one another in their amplitudes and half-widths, but not frequencies. Interestingly, we observed discrete Ca^{2+} flux frequencies in all three populations of astrocytic mitochondria in both the dorsolateral striatum (DLS) and the hippocampus (HPC). Compared to the DLS, astrocytic mitochondria in the HPC displayed an overall lower frequency of spontaneous Ca^{2+} fluxes. We also examined the effect of three neurotransmitter agonists: glutamate (excitatory neurotransmitter), SKF-38393 (D_1 receptor agonist) and quinpirole (D_2 receptor agonist) on mitochondrial Ca^{2+} fluxes in astrocytes from the DLS and HPC. Both brain regions showed robust changes in Ca^{2+} signal frequency for all three subpopulations of astrocytic mitochondria. We then utilized mitochondrial calcium uniporter (MCU) KO mice to assess the source of Ca^{2+} fluxes in astrocytic mitochondria. Surprisingly, MCU KO mice showed no reduction in Ca^{2+} signal parameters when compared to WT littermates, indicating that Ca^{2+} entry through MCU may not play a major role in astrocytic mitochondria. In summary, our data reveal significant morphological and functional differences in astrocytic mitochondria at a subcellular and circuit level. We also suggest that astrocytic mitochondria possess distinct routes for Ca^{2+} flux than mitochondria in other cell types. Future experiments will examine astrocytic mitochondrial Ca^{2+} signals in the context of aging and Parkinson's disease.

Disclosures: T.E. Huntington: None. **T. Patterson:** None. **M. Valle:** None. **R. Srinivasan:** None.

Poster

204. Astrocyte Biology: Cellular, Molecular, and Genetic Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 204.22/DP03/C12

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: B.11. Glial Mechanisms

Title: S100B alters functional properties in dopaminergic neurons via an extracellular mechanism

Authors: *E. BANCROFT, G. PANDEY, S. ZARATE, R. SRINIVASAN;
Neurosci. and Exptl. Therapeut., Texas A&M Univ. Col. of Med., Bryan, TX

Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disorder worldwide, with no known cure. Identifying therapeutic targets to treat PD has become an urgent medical need. A variety of neurological conditions such as Alzheimer's, PD, ALS, neurotrauma,

schizophrenia, and autism are associated with increased levels of the astrocyte marker S100B, to the extent that S100B in serum and CSF is utilized as a prognostic marker for these diseases. Previous studies have shown that S100B is secreted by astrocytes and can signal extracellularly via the receptor for advanced glycosylation end products (RAGE). Based on these ideas, we sought to understand if S100B plays an active role in the pathogenesis of PD. We stained midbrain sections of WT mice for S100B and TH to determine the extent of S100B expression in proximity to dopaminergic (DA) neurons. S100B-TH expression ratios are higher in the substantia nigra pars compacta (SNc) compared to the ventral tegmental area (VTA), suggesting that DA neurons in the SNc are generally exposed to more S100B compared to the VTA. To directly study effects of *extracellular* S100B on DA neuron function we turned to primary midbrain neuron-astrocyte co-cultures from ED14 mouse pups. Cultures were infected with TH-tdTomato and Syn-GCaMP6f AAVs at 14 DIV. We observed spontaneous Ca^{2+} events in TH- and TH+ neurons that were 2-fold greater in TH- compared to TH+ neurons. The VGCC bocker, diltiazem robustly inhibited Ca^{2+} events, while mibefradil, a T-type specific VGCC blocker, caused a partial inhibition of events, indicating. that the majority of Ca^{2+} events are mediated by L-type VGCCs. Acute exposure to 50 pM S100B caused an increase in frequency of Ca^{2+} events in TH+ neurons, while TH- neurons did not respond. To test if the observed effect of S100B was mediated by VGCCs, we bath applied diltiazem following exposure to S100B. Diltiazem inhibited the S100B mediated increase of Ca^{2+} events in TH+ cells, whikle mibefradil did not, suggesting that S100B may be altering the spontaneous activity of TH+ neurons via L-type VGCCs. Future studies will focus on validating S100B-VGCC interactions as a therapeutic target for treating early stage PD.

Disclosures: **E. Bancroft:** None. **G. Pandey:** None. **S. Zarate:** None. **R. Srinivasan:** None.

Poster

204. Astrocyte Biology: Cellular, Molecular, and Genetic Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 204.23/C13

Topic: B.11. Glial Mechanisms

Support: Vassar College Startup Funds to Lori A. Newman

Title: Effects of 17-β estradiol on spatial working memory and AQP4 expression

Authors: *J. J. MCINVALE[1], L. C. KUPER[1], S. L. LAMBERT[1], J. BONANNO[1], L. A. NEWMAN[2];
[1]Program in Neurosci. and Behavior, [2]Dept. of Psychological Sci., Vassar Col., Poughkeepsie, NY

Abstract: Astrocytes are increasingly recognized as key influencers of cognition, by sustaining CNS homeostasis and enabling complex communication within the tripartite synapse. Specifically, astrocytes highly express aquaporin-4 (AQP4), a bidirectional water channel which promotes water and ion homeostasis within the synapse and at the vasculature. 17-β estradiol has been shown to regulate various astrocyte functions that contribute to cognition through estrogen receptors such as glutamate recycling, and anti-inflammatory and neurotrophic factor release. As such, estradiol has direct implications for maintaining non-reactive astrocyte physiology, as well as exerting counteractive effects in CNS injury or disease. It is currently unclear if estradiol regulates AQP4 expression in healthy physiology, and to what extent this influences spatial working memory. To test this, female rats underwent bilateral ovariectomy to deplete circulating sex hormones. After ~2 weeks, vaginal smears were taken to assess whether systemic estradiol levels were low. Then rats received two successive subcutaneous doses of either sesame oil vehicle, low (4.5 µg/kg), or high dose (45 µg/kg) of 17-β estradiol at 48 and 24 hours before testing. A group of rats from each dose were tested using a delayed spontaneous alternation task to assess spatial working memory and sacrificed along with cagemate controls that did not receive any behavioral testing. Immunohistochemical analysis for AQP4 was done in the prelimbic cortex, hippocampus, and dorsolateral striatum. Behavioral results confirm previous literature that estradiol is critical for spatial working memory in female rats, with estradiol enhancing percent alternation 34% above oil ovariectomized control. Quantification of immunohistochemical results assessed changes due to training and changes in circulating estradiol levels. Future work should confirm that observed AQP4 staining is specific to astrocytes and investigate degree of AQP4 localization to the endfeet.

Disclosures: J.J. McInvale: None. **L.C. Kuper:** None. **S.L. Lambert:** None. **J. Bonanno:** None. **L.A. Newman:** None.

Poster

204. Astrocyte Biology: Cellular, Molecular, and Genetic Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 204.24/C14

Topic: B.11. Glial Mechanisms

Support: NRF 2016R1A6A3A04006478
MRC 2014R1A5A2009392

Title: Connexin43, an astrocytic gap junction protein, is selectively degraded by autophagy

Authors: *S. KIM[1], J. CHANG[2], S. LEE[1];
[1]Dept. of Anat., Hypoxia-Related Dis. Res. Center, Col. of Medicine, Inha Univ., Incheon, Korea, Republic of; [2]Dept. of Brain Sci. and Dept. of Biomed. Sci., Ajou Univ. Sch. of Med., Suwon, Korea, Republic of

Abstract: Cell-to-cell communications are emerging as new therapeutic targets in neurodegenerative disorders. Gap junctions are well-conserved intercellular communication systems mainly composed of connexins. Connexin43 (GJA1) is highly expressed in astrocytes, the main central nervous system cells coupled through gap junctions. Mice deficient in astrocytic *Gja1* show reduced synaptic plasticity and neuronal inactivation. In addition, depletion of *Gja1* in astrocytes reduces protein levels of Apoe, a well-known risk factor of Alzheimer's disease (AD), and impairs amyloid β phagocytosis. Although connexin43 is considered as a critical regulator of AD pathogenesis, the regulatory mechanism of connexin43 is not yet fully understood. Connexin43 exhibits remarkably short half-lives, and its degradation is responsible for autophagy, the intracellular mechanism for degradation of intracellular components in lysosomes. In order to identify the specific autophagic receptor of connexin43 for selective autophagy, we analyzed connexin43 protein levels in various autophagic receptor-knock out cell lines generated by a CRISPR-Cas9 system. We found that degradation of connexin43 is mediated by a specific autophagic receptor. The detailed mechanism of cx43 degradation will be shown in the presentation. Our results will help to develop ways to modulate connexin43 protein levels for treatment of AD patients.

Disclosures: S. Kim: None. **J. Chang:** None. **S. Lee:** None.

Poster

204. Astrocyte Biology: Cellular, Molecular, and Genetic Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 204.25/C15

Topic: B.11. Glial Mechanisms

Support: M280001-HH4059.

Title: The astrocyte neuron lactate shuttle modulates neuronal activity and sleep/wake cycles

Authors: *M. CHIACCHIARETTA, A. BRAGA, J. CLASADONTE, P. HAYDON; Neurosci., TUFTS Univ. Sch. of Med., Boston, MA

Abstract: Sleep plays a vital role in health and well-being and is critical for immune function, metabolism, learning and memory. We have recently shown that the astrocyte neuron lactate shuttle (ANLS) is required in the lateral hypothalamus to support orexinergic neuronal activity: impairment of this pathway silences these neurons and leads to excessive daytime sleepiness. In support of this notion we now demonstrate that in vivo delivery of the monocarboxylate transport inhibitor 4-CIN to the lateral hypothalamus similarly causes excessive sleepiness in the dark phase. Astrocytic connexin 43 (Cx43) is essential in providing lactate to adjacent neurons. In the lateral hypothalamus neurons are silent in Cx43 KO mice. We now demonstrate that brain wide deletion of Cx43 leads to a reduction in the power of slow wave activity (SWA) during NREM

sleep suggesting that the ANLS supports neuronal activity in the cortex as well as the lateral hypothalamus. Whole cell recordings from layer V cortical pyramidal neurons show reduced neuronal firing frequency that is accompanied by a hyperpolarization of the resting membrane potential. Taken together these results demonstrate that astrocytic Cx43 is essential for the ANLS and provides metabolic support that sustains neuronal activity. Impairments in this pathway lead to changes in sleep wake/cycles and in the power of SWA.

Disclosures: M. Chiacchiaretta: None. **A. Braga:** None. **P. Haydon:** None. **J. Clasadonte:** None.

Poster

204. Astrocyte Biology: Cellular, Molecular, and Genetic Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 204.26/C16

Topic: B.11. Glial Mechanisms

Support: MH-083728
NS050274

Title: Sex-dependent effects of astrocyte DISC1 knockdown on glutamate transporter and cognitive behaviors in mice

Authors: *A. V. SHEVELKIN[1,2], C. TERRILLION[2], V. MISHENEVA[2], Y. JOUROUKHIN[2], O. A. MYCHKO[2], J. A. CRAWFORD[2], S. H. KIM[2], D. FUKUDOME[2], A. SAWA[2], A. KAMIYA[2], M. V. PLETNIKOV[2];
[1]P.K.Anokhin Inst. Norm Physiol, Moscow, Russian Federation; [2]Johns Hopkins Univ., Baltimore, MD

Abstract: Background: Sex-dependent differences in astrocyte morphology and functions have been reported. However, only few studies reported sex-dependent effects on animal behavior after manipulation with astrocyte. Disrupted-in-Schizophrenia 1 (DISC1) is a gene that is disrupted by the balanced chromosomal translocation that is associated in a Scottish family with major psychiatric disorders. We evaluated whether decreased expression of DISC1 in astrocytes would impair learning and memory in mice in sex-dependent manner.
Methods: Male and female C57BL6 8-week-old mice were injected with the target (AAV1-GFAP::GFP-miR30-DISC1) (*Disc1* KD) or the control AAV1-GFAP::GFP-mir30-Ctrl (Ctrl) (control) vector in the dorsal hippocampus. 2-5 weeks later, we evaluated mouse hippocampus-dependent learning and memory followed by immunocytochemical studies of expression of glutamate transporters and synaptic markers within the astrocyte zones using Imaris software.
Results: Disc1 KD in astrocytes of the dorsal hippocampus produced no alterations in locomotor activity or anxiety but significantly decreased social preference and preference for a novel mouse

in female but not male mice. Additionally, DISC1 KD in astrocytes impaired performance in the Barnes maze and reduced cue-dependent freezing in trace fear conditioning of female but not male mice. DISC1 KD increased astrocyte density and decreased EAAT1 (Glast) expression by astrocytes of female but male mice. We also found reduced density of excitatory VGlut1+/PSD95+ synapses and increased density of inhibitory VGAT+/Gephyrin+ synapses in in female mice.Conclusion: Our findings indicate that Disc1 KD in the dorsal hippocampus produces sex-dependent changes in learning and memory, astrocyte morphology, expression of glutamate transporter and synaptic markers in astrocytes. The data suggest that estrogens could play a key role in modulating the astrocyte physiology to contribute to cognitive function in a sex-related manner.

Disclosures: **A.V. Shevelkin:** None. **C. Terrillion:** None. **V. Misheneva:** None. **Y. Jouroukhin:** None. **O.A. Mychko:** None. **J.A. Crawford:** None. **S.H. Kim:** None. **D. Fukudome:** None. **A. Sawa:** None. **A. Kamiya:** None. **M.V. Pletnikov:** None.

Poster

204. Astrocyte Biology: Cellular, Molecular, and Genetic Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 204.27/C17

Topic: B.11. Glial Mechanisms

Support: NIH R3734860

Title: A role for hypothalamic astrocytes in regulating gonadotropin-releasing hormone (GnRH) neuron activity and LH release

Authors: *C. H. VANACKER[1], S. M. MOENTER[1,2];
[1]Mol. and Integrative Physiol., [2]Intrnl. Medicine, and Obstetrics and Gynecology, Univ. of Michigan, Ann Arbor, MI

Abstract: GnRH neurons regulate fertility via episodic GnRH release, which triggers LH release. This episodic pattern suggests coordination among GnRH neurons but the source of this coordination remains unknown. GnRH neurons are surrounded by astrocytes, which can modulate neurotransmission and communicate over large distances. Prostaglandin E2 (PGE2), mainly produced by astrocytes in hypothalamus, increases GnRH neuron firing and LH levels in rodents. We hypothesized that astrocytes play a role in GnRH neuron coordination by modulating their activity. To test this, we injected AAV bearing DREADDs (designer receptor exclusively activated by designer drugs) and mCherry driven by the glial fibrillary acidic protein (GFAP) promoter, which is expressed primarily in astrocytes. Male GnRH-GFP mice were bilaterally injected in the medial preoptic area. 80.9% of infected cells were positive for the astrocyte marker S100β while only 11% expressed the neural marker NeuN (n=2). No mCherry

was detected in GnRH neurons. The DREADD utilized activates Gq signaling in the presence of clozapine-N-oxide (CNO). Intraperitoneal injection of CNO (0.3mg/kg) induced a marked and rapid increase in LH in mice with Dq-mCherry virus whereas no change was detected in mice with control virus expressing only mCherry (LH at first sample post CNO (10 min), Dq-mCherry, n=10, 3.48±0.39 ng/mL; mCherry, n=5, 0.47±0.11 ng/mL, $p<0.001$). One to three weeks later, extracellular recordings were used to monitor firing activity of GFP-identified GnRH neurons in brain slices from these same mice. CNO increased firing rate ≥50% in 7 of 8 GnRH neurons from Dq-mCherry injected mice (baseline 0.12±0.05 Hz, CNO 0.94±0.26 Hz, n=8, $p<0.0001$). In contrast, no change was observed in cells from mCherry controls (baseline 0.22±0.09 Hz, CNO 0.19±0.08 Hz, n=12). Preliminary data indicate that a mix of PGE2 receptor antagonists applied as a pretreatment blocked the CNO-induced increase in firing in 3 of 5 cells, suggesting PGE2 is at least in part involved in the increase in GnRH firing induced by the local activation of astrocyte Dq-signaling. These data provide evidence that altering signaling in astrocytes, specifically in the region containing GnRH neurons, affects GnRH neuron firing and LH release.

Disclosures: C.H. Vanacker: None. **S.M. Moenter:** None.

Poster

204. Astrocyte Biology: Cellular, Molecular, and Genetic Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 204.28/C18

Topic: B.11. Glial Mechanisms

Support: NIH grant R01AG034389
NIH grant R01NS095215
NSF grant 1615874

Title: Ceramide regulates interaction of Hsd17b4 with Pex5 and function of peroxisomes

Authors: *Z. Z. ZHU[1], J. CHEN[3], G. WANG[4], A. ELSHERBINI[5], L. ZHONG[4], X. JIANG[4], H. QIN[1], P. TRIPATHI[2], W. ZHI[6], S. SPASSIEVA[2], A. MORRIS[7], B. ERHARD[1];
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Abstract: The sphingolipid ceramide regulates beta oxidation of medium and long chain fatty acids in mitochondria. It is not known whether it also regulates oxidation of very long chain fatty acids (VLCFAs) in peroxisomes. Using affinity chromatography, co-immunoprecipitation

experiments, and proximity ligation assays we discovered that ceramide interacts with Hsd17b4, an enzyme critical for peroxisomal VLCFA oxidation and docosahexaenoic acid (DHA) generation. In HEK293T cells and astrocytes, Hsd17b4 is distributed to ceramide-enriched mitochondrial-associated membranes. Molecular docking and *in vitro* mutagenesis experiments showed that ceramide binds to the sterol carrier protein 2-like domain in Hsd17b4 adjacent to PTS1, the C-terminal signal for interaction with Pex5, a peroxin mediating transport of Hsd17b4 into peroxisomes. Inhibition of ceramide biosynthesis induced translocation of Hsd17b4 to peroxisomes, interaction of Hsd17b4 with Pex5, and upregulation of DHA. This data indicates a novel role of ceramide as molecular switch regulating interaction of Hsd17b4 with Pex5 and peroxisomal function.

Disclosures: **Z.Z. Zhu:** None. **J. Chen:** None. **G. Wang:** None. **A. Elsherbini:** None. **L. Zhong:** None. **X. Jiang:** None. **H. Qin:** None. **P. Tripathi:** None. **W. Zhi:** None. **S. Spassieva:** None. **A. Morris:** None. **B. Erhard:** None.

Poster

205. Mechanisms of Bi-Directional Glia-Neuron Communication

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 205.01/C19

Topic: B.11. Glial Mechanisms

Support: MOST 107-2320-B-039-060-MY3

Title: Astrocytes enhance neural synchrony after spreading depolarization

Authors: *N. ZHOU[1,2], D. WU[2];
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Abstract: Neural synchrony is crucial for functional integration of neuronal networks and its abnormality is highly associated with brain diseases. Under neurological conditions such as stroke or migraine, spreading depolarization (SD) occurs as a pathophysiological response to severe neuronal hyperexcitation. It is unclear whether and how SD affects neural synchronization in these disorders. We have recorded membrane potentials from pairs of hippocampal neurons and found that SD substantially enhanced membrane potential synchronization at low frequencies (<10 Hz). The increased low-frequency synchrony and excessive power results mostly from the synchronized membrane depolarization, which sustained for hundreds of milliseconds and shared similar properties with astrocyte-originated, NMDA receptor-mediated slow inward currents. Inhibition of sustained neuronal depolarization by NMDA receptor antagonists suppressed SD-induced increase in low-frequency synchrony and coherence. In the Inositol trisphosphate receptor 2 knockout ($IP_3R2^{-/-}$) mice that selectively inhibit astrocytic Ca^{2+}

signaling and gliotransmitter release, the SD-induced slow inward currents and enhancement of neural synchrony were inhibited. These results revealed that astrocyte-to-neuron gliotransmission plays an important role in neural synchrony and neural network dysfunction in SD-related disorders.

Disclosures: **N. Zhou:** None. **D. Wu:** None.

Poster

205. Mechanisms of Bi-Directional Glia-Neuron Communication

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 205.02/C20

Topic: B.11. Glial Mechanisms

Support: CIHR Project Grant (PJT-159832)
NSERC Discovery Grant (RGPIN-2015-05571)
NIPS Cooperative Study Program (17-129, 18-133, 19-126)

Title: Astrocytic processes associated with synapses to orexin neurons: 3-dimensional analysis using correlative light-electron microscopy

Authors: *K. SEMBA[1], C. BRIGGS[1], S. HATADA[2], S. DEURVEILHER[1], Y. KUBOTA[2];
[1]Dalhousie Univ., Halifax, NS, Canada; [2]Natl. Inst. Physiol Sci. (NIPS), Okazaki, Japan

Abstract: Sleep–wake cycles are regulated by the alternate activation of sleep- and wake-promoting neurons. We recently showed that astrocytes dynamically regulate excitatory transmission to wake-promoting orexin (ORX) neurons and sleep-promoting melanin concentrating hormone (MCH) neurons in the lateral hypothalamus via glutamate transport in a cell type-specific manner and according to sleep history (Briggs et al., 2018, J Neurosci). One possible mechanism for this synaptic plasticity is structural remodeling of perisynaptic astrocyte processes, such as protrusion and withdrawal. However, little is known about perisynaptic astrocytes associated with ORX or MCH neurons.
In this study, we conducted 3D reconstruction of ORX neurons using correlative light-electron microscopy (EM), to characterize the ultrastructure of ORX neurons and astrocytes associated with synapses on ORX neurons in rat. ORX neurons were identified using an orexin-A antibody with laser confocal microscopy. The same neurons were then identified in serial ultrathin sections obtained with automated tape-collecting ultramicrotomy (ATUM) and scanning EM. Depending on the orientation, dendrites could be followed up to 120 um from the soma.
ORX cell bodies and dendrites had very few spines. Synapses were frequently found both on cell bodies and along dendrites (effort underway to establish morphological criteria for characterizing presynaptic axon terminals, using correlative light-EM with VGluT1/2 and VGAT staining in additional sections). At virtually all of these synapses, astrocytic processes were located near the

cleft but with variable distances. Typically, the same astrocytic process approached multiple synapses with the same ORX neuron, as well as multiple synapses with non-ORX dendrites. Direct apposition between different parts of the same, and apparently-different, astrocytic process was very common, and direct apposition was also observed between two astrocytic cell bodies. These astrocytic appositions may represent gap junctions, which are known to occur frequently between astrocytes. Reconstruction of single astrocytes indicated that each astrocyte emits branching processes of complex morphologies that surround various neuronal elements (dendrites, axons) while also making contacts among themselves.
These preliminary results suggest that astrocytes play an important role in regulating synaptic transmission to ORX as well as other neurons in the lateral hypothalamus. Quantitative analysis of astrocyte-cleft relationships is underway to assess sleep history-dependent remodeling of astrocytic processes at synapses with ORX neurons.

Disclosures: K. Semba: None. **S. Hatada:** None. **S. Deurveilher:** None. **Y. Kubota:** None. **C. Briggs:** None.

Poster

205. Mechanisms of Bi-Directional Glia-Neuron Communication

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 205.03/C21

Topic: B.11. Glial Mechanisms

Support: Ainsworth Medical Research Innovation fund

Title: Neuromodulation of astrocytic K^+ clearance

Authors: *Y. BUSKILA, A. BELLOT-SAEZ, J. MORLEY;
Western Sydney Univ., Campbelltown, Australia

Abstract: Potassium homeostasis is a fundamental requirement for brain function. Therefore, effective removal of excessive K^+ accumulation from the synaptic cleft during physiological activity is paramount. Astrocytes, the most prevalent cell type in the brain, play a key role in K^+ clearance from the extracellular milieu using mechanisms such as net uptake and spatial buffering through the astrocytic network. However, the cellular mechanisms which affect this clearance process are still unknown. Recently we showed that alterations in the concentrations of extracellular potassium ($[K^+]_o$) or impairments of the astrocytic clearance mechanism effect the resonance and oscillatory behaviour of both individual and networks of neurons. These results showed that astrocytes have the potential to modulate network activity, however the cellular effectors that may affect astrocytic K^+ clearance process are still unknown. In this study, we have investigated the impact of neuromodulators, which are known to mediate changes in network oscillatory behaviour, on the astrocytic clearance process. Our results indicate diverse effects of

different neuromodulators on the astrocytic K^+ clearance, suggesting that neuromodulators work in parallel via both neurons and astrocytes to modify network activity and thus behaviour.

Disclosures: Y. Buskila: None. **A. Bellot-Saez:** None. **J. Morley:** None.

Poster

205. Mechanisms of Bi-Directional Glia-Neuron Communication

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 205.04/C22

Topic: B.11. Glial Mechanisms

Support: Rigeneration Next Post-doctoral Fellowship
NIH Grant NS096352
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NIH Grant DA040701

Title: Control of goal-directed actions by thrombospondin receptor α2δ-1

Authors: *F. ULLOA SEVERINO[1], C. SRIWORARAT[2], I. KIM[4], R. N. HUGHES[3], E. PETTER[3], H. H. YIN[3], C. EROGLU[2];
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Abstract: The learning and performance of goal-directed actions is a complex mechanism that involves the basal ganglia circuit with its cortical and thalamic afferent inputs. Alteration in this circuit can impair voluntary movements causing dystonia or bradykinesia, typical of neurodegenerative diseases such as Huntington`s or Parkinson's Disease. Recent studies on the role of astrocytes in brain circuits have suggested that astrocytes are involved in memory allocation, neuromodulation, as well as in the formation of stereotyped behaviors. However, little is known about the role of astrocytes in controlling goal-directed actions. Astrocytes control neuronal connectivity by signaling to neurons through the secretion of synapse modulating proteins. A pivotal synaptogenic astrocyte-secreted signal is Thrombospondin (TSP). TSP induces synapse formation via binding to its neuronal receptor, the calcium channel subunit α2δ1. Loss of α2δ1 during development results in a severe reduction in excitatory cortical synapses and alteration in dendritic spine structures. To determine if astrocyte-neuron signaling via this pathway is involved in control of goal-directed actions, we first trained adult wild-type (WT) mice to perform a lever pressing task with a food reward, to establish which brain regions are engaged during Fixed Ratio (FR) schedules. Expression of c-Fos, an immediate early gene and marker of neuronal activity, was used to map enhanced activity across brain regions. The presence of the immediate early gene c-Fos expression was used to map enhanced activity in

specific brain regions. Second, we used constitutive α2δ1 null (KO) mice and littermate age-matched WT and heterozygous mice to investigate possible impairment in goal-directed action learning and performance. Finally, we deleted α2δ1 in a circuit specific manner using a conditional allele, to overcome developmental deficits present in the complete KO, which enabled us to pinpoint the cortical regions involved in controlling goal-directed actions via α2δ1-signalling. Our results show that surprisingly α2δ1-signalling is not required for learning or performance phases of goal-directed actions. However, global or conditional ablation of α2δ1 cause persistence of goal-directed actions as shown by increased chunking of lever presses and persistence under progressive ratio or extinction (no reward) contingencies. Taken together, these results highlight the importance of α2δ1 in controlling complex behaviors and point out that astrocyte-neuron communications via TSP/ α2δ1 pathway as a contributor to behavioral flexibility.

Disclosures: F. Ulloa Severino: None. **C. Sriworarat:** None. **I. Kim:** None. **R.N. Hughes:** None. **E. Petter:** None. **H.H. Yin:** None. **C. Eroglu:** None.

Poster

205. Mechanisms of Bi-Directional Glia-Neuron Communication

Location: Hall A

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Program #/Poster #: 205.05/C23

Topic: B.11. Glial Mechanisms

Support: NRF Grant 2015R1A3A2066619
KIST Grant 2E26860
NRF Grant 2017R1A5A2015391

Title: Activation of astrocytic Gi protein-coupled receptor causes conditioned place preference

Authors: *M.-H. NAM[1], K.-S. HAN[1], J. LEE[1], W. WON[1], W. KOH[1], J. BAE[2], J. WOO[1], J. KIM[1], E. KWONG[1], T.-Y. CHOI[3], H. CHUN[1], S.-B. KIM[4], K. PARK[1], S.-Y. CHOI[3], Y. BAE[2], C. LEE[1];
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Abstract: Although the mechanism underlying how contextual fear conditioning causes avoidance of the context is well established, how positive emotional valence, such as pleasure, associated with a certain context causes preference of the context is poorly understood. Here we show that activation of astrocytic Gi protein-coupled receptors (Gi-GPCRs) drives conditioned place preference (CPP) by means of astrocyte-specifically expressed hM4Di and specific modulation of astrocytic mu-opioid receptor (MOR), an exemplar endogenous Gi-GPCR, in the

CA1 hippocampus. Long-term potentiation (LTP) induced by a subthreshold stimulation with the activation of astrocytic Gi-GPCR at the Schaffer collateral pathway accounts for the memory acquisition to induce CPP. This astrocytic MOR-mediated LTP induction is dependent on astrocytic glutamate released upon activation of the astrocytic MOR and the consequent activation of the presynaptic mGluR1. Our study reveals that the paradoxical transduction of inhibitory Gi-signaling into augmented excitatory synaptic transmission through astrocytic glutamate is critical for the acquisition of contextual memory for CPP.

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Poster

205. Mechanisms of Bi-Directional Glia-Neuron Communication

Location: Hall A

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Program #/Poster #: 205.06/C24

Topic: B.11. Glial Mechanisms

Support: The Research Council of Norway 249988
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Letten Foundation

Title: Astroglial Ca^{2+} signaling across sleep-wake states

Authors: *L. BOJARSKAITE[1], D. M. BJØRNSTAD[1], K. H. PETTERSEN[1], K. S. ÅBJØRSBRÅTEN[1], K. G. A. VERVAEKE[2], W. TANG[1], R. ENGER[1], E. A. NAGELHUS[1]; [1]Letten Ctr. and GliaLab, Div. of Physiology, Dep. of Mol. Med., [2]Vervaeke Lab, Div. of Physiology, Dep. of Mol. Med., Univ. of Oslo, Oslo, Norway

Abstract: Recent evidence indicates that astrocytes regulate brain states, sleep homeostasis and sleep-dependent brain waste clearance. However, the signaling mechanisms the astrocytes employ to mediate these functions remain largely unknown. Here we provide new insights into astrocytic and neuronal Ca^{2+} signaling in layer 2/3 of the barrel cortex during volitional awake behaviors (locomotion, whisking, quiet wakefulness) and natural sleep (NREM sleep, intermediate state, REM sleep) using dual-channel two-photon imaging and virally delivered genetically encoded Ca^{2+} indicators (rAAV-*GFAP*-GCaMP6f for astrocytes, rAAV-*SYN*-jRGECO1a for neurons). Mouse behavior and brain state were determined using an infrared camera, electrocorticography (ECoG) and electromyography (EMG). We report for the first time astrocytic Ca^{2+} signaling during natural sleep, and overall, astrocytic Ca^{2+} signaling is reduced

during sleep compared to wakefulness. We show that astroglial Ca^{2+} signals vary between sleep states and that astrocytic Ca^{2+} signals precede sleep to wake transitions. Moreover, our results indicate that astrocytic inositol triphosphate receptor 2-mediated Ca^{2+} signaling modulates sleep architecture in terms of sleep bout duration, ECoG power and sleep spindle frequency. We are currently quantifying the relationship between astrocyte Ca^{2+} signals, neuronal activity and ECoG. In conclusion, our data shows that astrocyte Ca^{2+} activity is largely behavioral state dependent and suggests that astrocyte Ca^{2+} signals are potential mediators of behavioral state shifts in the brain.

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Poster

205. Mechanisms of Bi-Directional Glia-Neuron Communication

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Topic: B.11. Glial Mechanisms

Support: NRF-2018M3C7A1056897
CRC-15-04-KIST
18-BR-03-02
NRF-2019R1A2C2003407

Title: Ultrasonic neuromodulation via astrocytic TRPA1

Authors: *S.-J. OH;
KIST, Seoul, Korea, Republic of

Abstract: Low-intensity, low-frequency ultrasound (LILFU) is the next-generation, non-invasive brain stimulation technology for treating various neurological and psychiatric disorders. However, the underlying cellular and molecular mechanism of LILFU-induced neuromodulation has remained unknown. Here we report that LILFU-induced neuromodulation is initiated by mechanical activation of TRPA1 channels in astrocytes. The Ca^{2+} entry through TRPA1 causes a release of gliotransmitters including glutamate through Best1 channels in astrocytes. The released glutamate activates NMDA receptors in neighboring neurons to elicit action potential firing. Our results reveal an unprecedented mechanism of LILFU-induced neuromodulation involving mechanosensitive TRPA1 as a unique sensor for LILFU and glutamate-releasing Best1 as a mediator of glia-neuron interaction. These discoveries should prove to be useful for optimization of human brain stimulation and ultrasonogenetic manipulations of TRPA1.

Disclosures: S. Oh: None.

Poster

205. Mechanisms of Bi-Directional Glia-Neuron Communication

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Topic: B.11. Glial Mechanisms

Support: CENTRO-01-0246-FEDER-000010
FCT project: PTDC/NEU-NMC/4154/2014
FCT project. POCI-01-0145-FEDER-031274
MIA Portugal

Title: Role of astrocytes on mouse hippocampal synaptic plasticity and memory

Authors: *P. M. AGOSTINHO[1,2,3], M. PEREIRA[1], I. AMARAL[1], C. LOPES[1], P. CANAS[1], R. A. CUNHA[1,2,3];
[1]Ctr. for Neurosciences and Cell Biol., Coimbra, Portugal; [2]Fac. of Medicine, Univ. of Coimbra, Coimbra, Portugal; [3]MIA-Portugal, Coimbra, Portugal

Abstract: Astrocytes can regulate the strength of synaptic connections between neurons, which constitutes the neurophysiological basis of learning and memory. We now compared the impact of acute and chronic astrocytic dysfunction in mouse hippocampal synaptic plasticity (long-term potentiation, LTP), and in memory, using a gliotoxin L-α-aminoadipate (L-AA), which was administrated *ex-vivo* (acutely) or *in vivo* (chronically). Slices exposed for 2 hours to L-AA (100 µM) displayed a lower LTP amplitude (43.4±5.3% *vs*. 74.3±8.2% in control, n=6), without changes of basal synaptic transmission in Schaffer fiber-CA1 pyramid synapses. This condition of acute L-AA exposure also caused reactive astrogliosis, as heralded by the increased glial fibrillary acidic protein (GFAP) immunoreactivity and by alterations in the complexity and length of ramifications of 3D-reconstituted astrocytes. To assess the impact of this gliotoxin on memory, we administrated intracerebroventricularly (icv) L-AA (1 µmol/4 µl) for three consecutive days in cannulated C57BL/6 adult mice. Icv L-AA mice had a significant ($p<0.05$, n=9-10) reduction of memory performance, in the object displacement and in modified Y-maze tests, when compared with icv-saline mice (control). These impairments in hippocampal-dependent memory were accompanied by a significant ($p<0.05$, n=5) reduction of LTP amplitude (52.4±6.2% compared to control: 77.0±8.5%). However, in contrast to that observed for acute L-AA exposure, chronic L-AA administration significantly ($p<0.05$) decreased by circa 10% the number of GFAP-positive cells in different hippocampal subregions, and also reduced the levels of astrocytic proteins (GFAP and connexin 43). Altogether, these studies uncovered the time course of changes triggered by L-AA in the hippocampus and contributed to establish that astrocytes are crucial to regulate hippocampal synaptic plasticity and memory, which might

be relevant to design potential therapeutics towards brain disorders associated with memory decline.
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Poster

205. Mechanisms of Bi-Directional Glia-Neuron Communication

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 205.09/C27

Topic: B.11. Glial Mechanisms

Title: Retinal inputs signal through astrocytes to recruit interneurons into mouse visual thalamus

Authors: *R. SOMAIYA[1], J. SU[1], N. CHARALAMBAKIS[2], A. MONAVARFESHANI[1], U. SABBAGH[1], W. GUIDO[2], M. A. FOX[1];
[1]Virginia Tech., Roanoke, VA; [2]Univ. of Louisville, Louisville, KY

Abstract: The lateral geniculate complex includes a number of retinorecipient thalamic nuclei that play important roles in receiving, processing, and relaying image- and non-image forming visual information. Recent studies have revealed that neonatal innervation of these regions by retinal ganglion cell axons plays an instructive role in the postnatal development and maturation of thalamic circuits. For example, surgical or genetic removal of retinal inputs at birth impairs the recruitment of local GABAergic interneurons into visual thalamus. Here we sought to identify and characterize the molecular mechanisms that underlie retinal input-dependent interneuron migration into visual thalamus. To begin to address this issue, we explored transcriptomic changes in neonatal mouse visual thalamus lacking retinal input. Using microarray analysis and *in situ* hybridization (ISH), we discovered that the expression of Fibroblast Growth Factor 15 (FGF15) in visual thalamus is dependent upon the presence of retinal inputs. To test whether FGF15 was required for interneuron recruitment into visual thalamus, we examined thalamic development in *Fgf15*$^{-/-}$ mutant mice. In these mutants, we observed a significant reduction in GABAergic interneurons both by ISH and by crossing these mutant mice to *Gad67-GFP* reporter mice (in which thalamic GABAergic interneurons are labelled with GFP). We hypothesized that retinal inputs induced FGF15 expression in retinorecipient neurons (i.e. thalamocortical relay neurons). To test this, we performed ISH to detect *Fgf15* mRNA in reporter mice that label distinct populations of cells in the visual thalamus. Surprisingly, our data revealed that FGF15 is generated by thalamic astrocytes and not neurons, suggesting a novel role for astrocytes in thalamic development. Taken together, these

results suggest the existence of novel axon-glia-neuron signaling pathway that underlies subcortical visual circuit formation.

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Poster

205. Mechanisms of Bi-Directional Glia-Neuron Communication

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Topic: B.11. Glial Mechanisms

Support: CIHR Project Grant PJT-159779
CIHR Operating Grant MOP-259183
Plum Foundation

Title: Immunoconfocal analysis of microglia associated with sleep/wake-regulatory orexin and melanin-concentrating hormone neurons in the rat lateral hypothalamus

Authors: *S. HALL[1], S. DEURVEILHER[1], K. SEMBA[1,2,3];
[1]Med. Neurosci., [2]Psychology & Neurosci., [3]Psychiatry, Dalhousie Univ., Halifax, NS, Canada

Abstract: The lateral hypothalamus (LH) houses several intermingled populations of sleep/wake-regulatory neurons, including wake-promoting orexin/hypocretin (ORX) neurons and sleep-promoting melanin-concentrating hormone (MCH) neurons. We recently found that chronic sleep restriction for 4 days increased markers of neuronal activation (Hall et al., 2017) and the number of immunohistochemically-identified microglia (Hall et al., In revision) in the LH and other brain regions in rats. Microglia are the innate immune cells of the brain, and recent evidence recognizes them as significant contributors to synaptic circuit structuring and plasticity. Whether microglia interact with sleep/wake-regulatory LH neurons and, if so, whether chronic sleep restriction alters these interactions are unknown.
As a first step, in this pilot study we assessed the structural relationship of microglia with ORX and MCH neurons in the LH under physiological conditions in adult male rats (n=3) using immunoconfocal microscopy. Microglia, ORX and MCH neurons were identified using antibodies against Iba1, orexin-A, and pro-melanin-concentrating hormone, respectively, and Nissl stain was used to identify all brain cells.
Microglia were fairly evenly distributed throughout the LH. Microglia processes frequently contacted the cell bodies of ORX and MCH neurons, as well as Nissl+ cell bodies. The cell bodies of some microglia were in contact with ORX, MCH and other (Nissl+) neuronal cell bodies; such microglia are referred to as perineuronal “satellite” microglia. On average, satellite microglia represented 46% of total microglia in the LH and were found in contact with 4% and

7% of ORX and MCH neurons, respectively. Both satellite and non-satellite microglia in the LH appeared ramified, with small, typically round cell bodies and numerous branching processes. Satellite microglia tended to have a slightly elongated cell body, as they lay extending along the soma of the contacted neuron. A similar proportion of satellite microglia (46%) was observed in the prefrontal cortex, another brain region in which increased microglia numbers were found after chronic sleep restriction.
In conclusion, the close structural relationship of microglia, in particular satellite microglia, with ORX and MCH neurons suggests that microglia may modulate the activity of these cells either directly or through modulation of their synaptic inputs, to regulate sleep behaviour and sleep homeostasis. We are currently investigating whether microglial interactions with the sleep/wake-regulatory neurons are altered by chronic sleep restriction.

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Poster

205. Mechanisms of Bi-Directional Glia-Neuron Communication

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 205.11/C29

Topic: B.11. Glial Mechanisms

Support: NIH Grant DA35805

Title: Limited astrocytic ensheathment of axospinous synapses in the rat nucleus accumbens core

Authors: S. R. SESACK[1], P. IYENGAR[1], G. BURNET[1], S. BIAGIOTTI[1], J. BALCITA-PEDICINO[1], Y. H. HUANG[2], ***Y. DONG**[1];
[1]Neurosci., [2]Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Astrocytes regulate homeostasis at glutamate synapses, and this regulation is often targeted by drugs of abuse to reshape synaptic transmission. The canonical tripartite synapse involves bidirectional communication between neuronal and glial elements at excitatory inputs onto dendritic spines in brain regions containing spiny neurons. Surprisingly, ultrastructural studies of naïve rats indicate considerable variability in the extent to which astrocytes ensheath axospinous synapses in the hippocampus, cortex, and cerebellum. We sought to examine these associations in the nucleus accumbens (NAc), a region important for goal-directed behaviors and strongly implicated in substance abuse disorders. Archival tissue containing the NAc core from 3 adult male rats was examined by electron microscopy. 78 axospinous synapses were reconstructed in serial sections using *Reconstruct™* software (Witcher et al., 2007 *Glia* 55:13; Harris et al., 2015 *Sci Data* 2:150046). Of these synapses, 34 (44%) had no astrocytic profiles in contact with the synaptic cleft; most also had no glial contact with the plasma membrane of the

axon or spine. For the remaining 44 synapses, the average extent to which astrocytes made close contact with the synaptic cleft was 33%, with a range of 8-75%. Axons with some glial association were contacted by astrocytes over 2-57% of their surface area, with a mean of 15%. Dendritic spines with some glial coverage had an average of 17% of their surface area in contact with astrocytes and a range of 4-59%. The extent to which astrocytes covered the surface area of axons and spines was not significantly different. Across all animals, the coefficients of variation for astrocytic coverage of synapses, axons, and spines were 45%, 87%, and 79%, respectively, while the values for comparing the animal means were 9%, 11%, and 18%, respectively. This consideration suggests that the largest source of variability was within animals and not inter-animal. This study marks the first ultrastructural examination of astrocytic ensheathment of axospinous synapses in the rat striatal complex. The limited extent to which astrocytes contact glutamate synapses in the NAc core is similar to the hippocampus, for which 43% of synapses also lack glial coverage and for which the synapses that are contacted have less than half the synaptic surface area covered by astrocytes (Ventura & Harris, 1999 *J Neurosci* 19:6897). Whether glial contact of synapses in the NAc core is comparable to the NAc shell or dorsal striatum remains to be determined. The findings have important implications for understanding morphological changes in NAc astrocytes in response to drugs of abuse.

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Poster

205. Mechanisms of Bi-Directional Glia-Neuron Communication

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Topic: B.11. Glial Mechanisms

Support: NIH Grant R21 MH107029
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Title: Modulation of astrocytic Ca^{2+} activity in the motor cortex with learning

Authors: *P. RAGUNATHAN, A. DUNAEVSKY;
Dept. of Neurolog. Sci., Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Motor skill learning induces changes in synaptic structure and function in the primary motor cortex. Astrocytes form an integral part of the nervous system architecture, are in intimate structural relationship with synapses and have been shown to respond to various neurotransmitters and neuromodulators by increases in intracellular calcium levels. We have earlier shown that normal astrocytic activity and Ca^{2+} signaling during a reaching task are necessary for motor skill learning. However, it is not known whether motor learning modulates

astrocyte Ca^{2+} activity and if the extent of Ca^{2+} activity in the astrocytic processes promotes dendritic spine stability. Here, we perform two-photon imaging of astrocytic Ca^{2+} activity and structural imaging of dendritic spines on awake mice following training on a forelimb reaching task. The temporal development of Ca^{2+} activity in astrocytic processes with learning and the relationship between astrocytic Ca^{2+} activity and structural synaptic plasticity are examined by repeated in vivo imaging. Our results show that motor skill learning results in enhanced Ca^{2+} signaling in the trained hemisphere. Understanding the role of astrocytic Ca^{2+} signaling is important as this could be one of the mechanisms by which astrocytes participate in the cellular processes of learning.

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Poster

205. Mechanisms of Bi-Directional Glia-Neuron Communication

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Topic: B.11. Glial Mechanisms

Support: NIH Grant NS102822
SUNY Albany
SUNY Research Foundation

Title: Circadian control of excitatory synaptic transmission in the hippocampus

Authors: *J. MCCAULEY, M. A. PETROCCIONE, G. TODD, N. AFFINNIH, S. ZAHID, A. SCIMEMI;
SUNY Albany, Albany, NY

Abstract: One of the most fascinating questions in neuroscience is how the brain shapes our cognitive skills in response to changing internal and external stimuli. In vertebrates and invertebrates, different cell types in the brain display plastic behaviors, because they undergo structural and functional modifications over a wide range of time scales. Previous work in the hippocampus showed that the magnitude and incidence of long-term potentiation, a proposed cellular substrate for memory formation, shows circadian oscillations. Accordingly, this form of plasticity is enhanced between ZT0-12 compared to ZT12-24. What accounts for these changes? By using patch-clamp electrophysiology, fluorescence in situ hybridization, imaging and protein retention expansion microscopy, we show that neurons and astrocytes in the hippocampus are differently affected by circadian rhythmicity. These changes shape the ability of hippocampal pyramidal cells to integrate synaptic inputs and express long-term changes in synaptic strength. Together, these findings highlight previously unknown forms of cellular remodeling that can contribute to shape the cognitive skills of living organisms.

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Poster

205. Mechanisms of Bi-Directional Glia-Neuron Communication

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Title: Noradrenergic modulation of microglial dynamics and synaptic plasticity

Authors: *R. STOWELL[1], G. O. SIPE[2], A. K. MAJEWSKA[3];
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Abstract: Microglia have long been recognized for their role as the resident immune cell of the central nervous system. However, in addition to their immunological function, microglia have recently come to be recognized as integral participants in synaptic pruning during development as well as synaptic plasticity during both development and adulthood. In the absence of any pathological perturbations microglia are very dynamic cells with a complex arbor of processes that rapidly extend and retract to survey the brain parenchyma. Importantly, previous *in vivo* monitoring of microglial dynamics has been done in anesthetized preparations and thus it remains poorly understood how microglia behave in the awake, alert brain. Here, utilizing chronic cranial windows and *in vivo* two-photon microscopy, we observe reduced parenchymal surveillance and focal tissue injury responsiveness by microglia in awake mice. We find that direct pharmacological activation of microglial beta-2 adrenergic receptors, utilizing the beta-2 adrenergic agonist clenbuterol, similarly reduces surveillance and response to focal tissue injury, suggesting that noradrenergic receptor signaling in the awake state may be a critical regulator of microglial dynamics. We further demonstrate that activation of beta-2 adrenergic receptors reduces microglial interactions with dendritic spines, suggesting norepinephrine could serve as a negative regulator of microglia-synapse interactions. Finally, we find that in addition to the robust regulation of microglial process dynamics, noradrenergic signaling through the beta-2 adrenergic receptor inhibits ocular dominance plasticity during the mouse visual critical period.

We show that this inhibition of plasticity is specific to microglial beta-2 adrenergic receptors by conditionally knocking out beta-2 receptors in microglia. Our work identifies the beta-2 adrenergic receptor on microglia as a key regulator of microglial process dynamics, injury response, and microglial-neuron interactions during experience dependent plasticity. Importantly, we also demonstrate that microglial physiology is regulated by brain state, suggesting that like neurons and astrocytes, microglia may undergo important physiological changes between sleep-like and awake conditions. A better understanding of the neuromodulatory cues which guide microglia-neuron interactions may lead to a more comprehensive view of how dysregulation of these signals can lead to neurological disease.

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Poster

205. Mechanisms of Bi-Directional Glia-Neuron Communication

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Program #/Poster #: 205.15/C33

Topic: B.11. Glial Mechanisms

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Gerald Choa Neuroscience Centre (7105306)

Title: Astrocyte hypertrophy and increased tripartite formation in motor cortex after training

Authors: *Y. KE, Y.-N. ZHENG, X.-F. YANG, L.-T. GENG, W.-H. YUNG;
The Chinese Univ. of Hong Kong, Shatin, Hong Kong

Abstract: Emerging evidence in past decades supports that astrocytes play fundamental roles in regulating synaptic transmission, thereby influences overall plasticity of neuronal networks. Despite this potential function of astrocytes, little is known about their role of in the formation of motor memory in the motor cortex. Based on a forelimb food pellet-reaching task, we investigated if cortical astrocytes can undergo structural changes during acquisition of new motor skills. We found an increase in arborization in astrocytes of the trained motor cortex. To substantiate this finding, we performed modified Golgi-Cox staining and confirmed that soma size of astrocytes was increased after training while the density of the astrocytes was unchanged. Furthermore, by using electron microscopy we found that astrocytic volume was increased after motor training. To probe further the possible participation of astrocytes in training, based on double immunofluorescence staining of synaptophysin, a presynaptic marker, and phospho-ezrin, which is expressed in peripheral astrocyte process we found that not only phospho-ezrin was increased in the trained motor cortex but the density of colocalized puncta was also increased, implicating increased tripartite formation due to training. Together, our findings suggest that the

interaction between neurons and astrocytes is enhanced with motor training, manifested as hypertrophy of astrocyte occurring along with increased tripartite formation.

Disclosures: **Y. Ke:** None. **Y. Zheng:** None. **X. Yang:** None. **L. Geng:** None. **W. Yung:** None.

Poster

205. Mechanisms of Bi-Directional Glia-Neuron Communication

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 205.16/C34

Topic: B.11. Glial Mechanisms

Support: The Research Council of Norway Grant #249988
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The South-Eastern Norway Regional Health Authority Grant #2016070
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The Letten Foundation

Title: Event based tool for assessing astroglial Ca^{2+} signal diversity

Authors: *D. M. BJØRNSTAD[1], K. S. ÅBJØRSBRÅTEN[2], L. BOJARSKAITE[4], K. H. PETTERSEN[3], E. A. NAGELHUS[2], R. ENGER[2];
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Abstract: Astrocytic Ca^{2+} signals in awake behaving mice are exceedingly rich and challenging to interpret. In part because they range from frequent, localized activity in subcellular compartments to less frequent, large and synchronized events that affect most of the astrocytes in the field-of-view. Currently, the research field is hampered by lack of a standardized way to extract and characterize such astrocytic Ca^{2+} signals. Conventional analyzes involve manually segmenting regions-of-interests representing subcellular compartments, but this method is prone to human bias and gives rise to both false-positive and false-negative results. We have developed an activity-based algorithm that in an unsupervised fashion extracts astrocytic Ca^{2+} signals from two-photon microscopy images. The algorithm comprises three-dimensional filtering and noise-based thresholding on individual pixels over time to detect fluorescent events. Connecting active pixels in space and time results in 'regions-of-activity' that can subsequently be combined with conventional, manually selected regions-of-interest or analyzed separately.This approach reveals that manually segmented regions-of-interests fail to capture both spatial and temporal aspects of the underlying astroglial Ca^{2+} signals. Our preliminary data also suggests that previously reported complex astroglial Ca^{2+} signaling motifs largely stem from spatiotemporal integration of multiple single peak events. Our algorithm will be a valuable tool for investigators assessing complex astroglial Ca^{2+} signaling in awake behaving animals. The algorithm could also prove

useful for quantifying other types of fluorescent events (e.g. extracellular glutamate levels by the glutamate sensor iGluSnFR).

Disclosures: D.M. Bjørnstad: None. **K.S. Åbjørsbråten:** None. **L. Bojarskaite:** None. **K.H. Pettersen:** None. **E.A. Nagelhus:** None. **R. Enger:** None.

Poster

205. Mechanisms of Bi-Directional Glia-Neuron Communication

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Program #/Poster #: 205.17/C35

Topic: B.11. Glial Mechanisms

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Scientific Prize Gustave Boël - Sofina Fellowship
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Scientific Fund Willy Gepts - UZ Brussel

Title: Validation of chemogenetic Gq-mediated astrocyte activation and its effects on synaptic transmission

Authors: *Y. VAN DEN HERREWEGEN[1], D. DE BUNDEL[1], A. VAN EECKHAUT[1], Z. A. BORTOLOTTO[2], I. SMOLDERS[1];
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Abstract: Epilepsy and neurodegenerative diseases often impact cognition as a primary or comorbid condition. Adequate treatment is still lacking and therefore novel strategies are necessary to tackle this issue. Increasing evidence shows that astrocytes play a key role in tuning neuronal activity and active brain functioning. Recent studies have found improvements in spatial and contextual memory upon chemogenetic Gq-mediated astrocyte activation. This finding was supported by the observation that Gq-mediated astrocytic activation induces long-term potentiation (LTP) in CA1 and increases in astrocytic Ca^{2+} events upon hM3Dq-ligand (CNO) application. This chemogenetic-based strategy has proven to be a promising tool to understand the role of astrocytes in memory acquisition, moreover, it might pave the way to appoint astrocyte modulation as a new treatment strategy for cognitive dysfunction. To validate DREADD-functionality, *ex vivo* Ca^{2+} imaging was performed. Acute coronal hippocampal slices were prepared from 11-13 weeks old male, C57Bl6/J mice, previously injected with viral vectors driving DREADD-hM3Dq expression in their astrocytes or the control vector. Increases in Ca^{2+} events during CNO application in Gq-transfected slices were observed, which returned to baseline after wash-out. Subsequently, as LTP is a crucial mechanism underlying learning and

memory, we performed extracellular field recordings in CA1 upon Schaffer collateral stimulation. After 10 min of CNO (10 μM) application no significant differences between Gq and control slices were observed ($p = 0.51$, $n = 4$). Nevertheless, significant increases in fEPSP amplitude were found 20 min after wash-out ($121.62 \pm 5.45\%$, $p = 0.008$, $n = 4$) for Gq-DREADD transfected slices, while no changes were observed in control slices (98.76 ± 2.18 %, $n = 4$). Our results are in line with literature (Adamsky *et al*., 2018). Now we are characterizing *in vivo* the effect of astrocyte modulation on spatial learning and memory in an animal model of epilepsy by use of the Barnes Maze. This experiment is still ongoing but we expect to gather additional information about the effects of astrocyte modulation of spatial memory in animals with established cognitive dysfunction. Although several studies have demonstrated that astrocytes influences memory function, further research is required to gain insights in the specific effects of astrocyte activation on memory in animal models for epilepsy and neurodegenerative disorders. Additionally, the roles of astrocytic Ca^{2+} and other second messengers in these memory processes are yet to be unveiled and will potentially boast the development of new therapeutic strategies.

Disclosures: **Y. Van Den Herrewegen:** None. **D. De Bundel:** None. **A. Van Eeckhaut:** None. **Z.A. Bortolotto:** None. **I. Smolders:** None.

Poster

205. Mechanisms of Bi-Directional Glia-Neuron Communication

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 205.18/C36

Topic: B.11. Glial Mechanisms

Support: NARSAD Grant 27202

Title: Expansion microscopy of mouse prefrontal cortex reveals microglial engulfment of synaptically targeted intrabodies

Authors: *T. JINADASA, K. LIU KOT, R. PHADKE, A. COMER, S. MAI, F. HAUSMANN, A. CRUZ-MARTIN;
Boston Univ., Boston, MA

Abstract: Brain development involves a sequence of highly orchestrated steps. Several lines of evidence suggest that microglia-neurons interactions are critical for shaping the developmental wiring of the brain. In particular, studies have shown that microglia might directly regulate synaptic refinement through activity-dependent phagocytosis of synaptic material and secretion of factors. In microglia, phagocytosed materials is trafficked to lysosomes and targeted for degradation. Using confocal imaging and expression of genetically engineered intracellular antibodies against PSD-95 (FingRs) in cortical neurons we previously showed that during early

development this postsynaptic protein is localized to microglia lysosomes, suggesting that microglia can regulate brain wiring by phagocyticing synaptic material. To gain more insights into the biology of microglia lysosome activity and improve the resolution and more precisely localize microglial lysosomes and their content we employed expansion microscopy (ExM). Using these techniques, both lateral and axial resolution was improved between 4-5 fold and synaptically tagged material from excitatory synapses was observed within the lysosomes of microglia in the prefrontal cortex of mice.

Disclosures: **T. Jinadasa:** None. **K. Liu Kot:** None. **R. Phadke:** None. **A. Comer:** None. **S. Mai:** None. **F. Hausmann:** None. **A. Cruz-Martin:** None.

Poster

205. Mechanisms of Bi-Directional Glia-Neuron Communication

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 205.19/C37

Topic: B.11. Glial Mechanisms

Support: NSERC Grant 195814317

Title: Neuromodulator-mediated effects on somatosensory adaptation are associated with changes in extracellular potassium clearance

Authors: C. A. WOTTON, ***L. K. BEKAR**;
Univ. of Saskatchewan, Saskatoon, SK, Canada

Abstract: It is well established that neuromodulators affect cortical frequency transmission. Additionally, a recent study demonstrated that application of a cocktail of neuromodulators to the cortex increased extracellular potassium ($[K^+]_e$) and changed its uptake. Given that altering extracellular potassium has the potential to alter neuronal excitability (by changing ion driving forces and depolarizing/hyperpolarizing neurons), we set out to test the hypothesis that neuromodulators use changes in $[K^+]_e$ to produce changes in frequency transmission using extracellular field and simultaneous K^+ ion-selective microelectrode recordings in acutely isolated somatosensory cortical mouse brain slices. A 10-pulse stimulation protocol (20 Hz), to assess adaptation/habituation, was repeated every two minutes throughout application of the different neuromodulators with/without various pharmacological agents. Results indicate that the neuromodulators differentially affect adaptation (frequency transmission). Serotonin and norepinephrine decrease, whereas acetylcholine increases adaptation/habituation. Interestingly, these effects were associated with serotonin and norepinephrine showing a decrease in the evoked response decay tau, while acetylcholine did not. To address the role of the Na^+/K^+ ATPase and K^+ inward rectifiers in neuromodulator responses on $[K^+]_e$, ouabain and Ba^{2+} were applied before administration of the various neuromodulators, respectively. In support of

extracellular potassium involvement in neuromodulator effects, we found that differential effects on adaptation were associated with differential effects on K^+ regulation. Interestingly, neuromodulator-mediated net effects on baseline $[K^+]_e$ were dramatically different in the absence of neural activity. In the absence of neural activity (100 nM tetrodotoxin), changes in baseline $[K^+]_e$ reflected changes in the decay tau of evoked $[K^+]_e$ responses. This study highlights a novel mechanism (extracellular potassium regulation) through which neuromodulators can alter cortical networks in a rapid and robust manner. It also indicates a possible role for astrocytes in neuromodulator-mediated effects given that astrocytes are the main mechanism through which K^+ homeostasis is maintained. These results have implications for attention and perception behaviors that rely on the principle of adaptation/habituation.

Disclosures: C.A. Wotton: None. **L.K. Bekar:** None.

Poster

205. Mechanisms of Bi-Directional Glia-Neuron Communication

Location: Hall A

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Program #/Poster #: 205.20/C38

Topic: B.11. Glial Mechanisms

Support: R01 HL128066
R01 MH081935
R01 DA017392
F31 MH109267

Title: Loss of the microglial protein AIF1/Iba1 affects synaptic structure-function, cognition and sociability

Authors: *S. NANDI, P. J. LITUMA, P. E. CASTILLO, N. E. S. SIBINGA;
Albert Einstein Col. of Med., Bronx, NY

Abstract: Growing evidence indicates that microglia play roles beyond classical phagocytosis of dead cells. These functions relate to synaptic formation, pruning, transmission, and plasticity, and thereby are expected to have important effects on behavior. Moreover, microglia have been implicated in neurodevelopmental and psychiatric conditions. To date, efforts to understand the role of microglia in synaptic function and behavior have utilized pharmacological agents to inhibit function or to cause microglial cell death. These strategies have distinct limitations- lack of microglial specificity, or rapid repopulation of cells after depletion accompanied often by significant astrogliosis, respectively- that complicate interpretation of results. Allograft inflammatory factor-1 (AIF1, a.k.a. Iba1) is a 17 kDa Ca^{2+} binding cytosolic protein expressed in microglia. While AIF1/Iba1 is widely used as a marker of microglia, and *in vitro* experiments link AIF1/Iba1 to cytoskeletal remodeling and phagocytosis, its effects on microglial and brain

functions *in vivo* remain largely unknown. Using global AIF1/Iba1 deficiency (*Aif1*$^{-/-}$) in mice, we report that AIF1/Iba1 is required for microglial activation/function, as assessed by morphology and expression of markers of activation (n=5). By combining electrophysiology in acute hippocampal slices with imaging studies, we found that loss of AIF1/Iba1 reduces both excitatory synaptic transmission and dendritic spines in the CA1 area of juvenile mice (n=4-6). Interestingly, adult *Aif1*$^{-/-}$ mice showed improvements in hippocampal-dependent tasks such as novelty-based exploration and object recognition memory, but also an impairment in sociability and an increase in repetitive behavior (n=16-21). Anxiety-like behavior and the preference for social novelty were, however, normal in adult *Aif1*$^{-/-}$ mice. Unexpectedly, preliminary results show that loss of AIF1/Iba1 enhances bioavailability of monoamine neuromodulators in the adult brain presumably by reducing their metabolism, suggesting a neuromodulator clearance function for microglia (n=3-4). In addition, loss of AIF1/Iba1 increased new-born neurons in the dentate gyrus of adult mice without causing reactive astrogliosis (n=3). In summary, our findings demonstrate multiple roles of AIF1/Iba1 in development and adulthood that can affect behavior, and further highlight the importance of AIF1/Iba1-directed microglia-neuron communication during early postnatal development. Investigators were blinded for all manipulations. Student's t or ANOVA with implementation of multiple comparison tests were performed.

Disclosures: S. Nandi: None. **P.J. Lituma:** None. **P.E. Castillo:** None. **N.E.S. Sibinga:** None.

Poster

205. Mechanisms of Bi-Directional Glia-Neuron Communication

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 205.21/C39

Topic: B.11. Glial Mechanisms

Support: MH113780
AA025128

Title: Revealing constraints on behavioral state-dependent astroglia calcium dynamics through simultaneous monitoring of calcium dynamics in neuromodulatory terminals

Authors: *E. LIM[1], A. M. SALINAS[2], X. XHU[1], M. PAUKERT[1];
[1]Cell. and Integrative Physiol., [2]Pharmacol., Univ. of Texas Hlth. San Antonio, San Antonio, TX

Abstract: In addition to performing critical supportive functions, astroglia exhibit an arousal-associated calcium response that is characterized by an initial synchronized global calcium rise that can vary among astrocytes from trial to trial. The source of variability in individual astroglia calcium responses is unknown. Two possible explanations are variable release of neuromodulator and differential abilities of astrocytes to produce a calcium rise. To test these models, calcium dynamics in astrocytes and noradrenergic terminals were monitored

simultaneously in mouse primary visual cortex during locomotion using *in vivo* two photon imaging. A green genetically encoded calcium indicator (GECI) GCaMP6f was targeted to noradrenergic neurons by specific Cre recombinase expression, and a red GECI driven by an astroglia-specific promoter was virally delivered. Our preliminary data show that locomotion induces calcium elevations in noradrenergic terminals consistent with the predicted release of norepinephrine and support the feasibility of simultaneous monitoring of astrocyte and neuromodulatory terminal calcium dynamics in mice during locomotion. Insight into constraints on astroglia calcium dynamics will inform the interpretation of astroglia activity in more complex and naturalistic behavior.

Disclosures: **E. Lim:** None. **A.M. Salinas:** None. **X. Xhu:** None. **M. Paukert:** None.

Poster

205. Mechanisms of Bi-Directional Glia-Neuron Communication

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 205.22/C40

Topic: B.11. Glial Mechanisms

Support: MH113780
AA025128

Title: Dissecting the mechanisms underlying astroglia Ca^{2+} activation

Authors: *A. M. SALINAS[1], M. PAUKERT[2];
[2]Cell. and Integrative Physiol., [1]UT Hlth. San Antonio, San Antonio, TX

Abstract: Astroglia, traditionally known for their supportive properties in the central nervous system, have recently been implicated in more active, regulatory roles in the brain. One of those roles includes their involvement in brain-state dependent signaling in awake, behaving animals. Robust astroglia Ca^{2+} activation occurs through the release of norepinephrine from locus coeruleus neurons following a transition from a resting to an active brain state. However, the temporal and spatial properties of global astroglia Ca^{2+} dynamics and receptor involvement subsequent to such a state transition-induced Ca^{2+} transient remain not well understood. In order to address this gap in knowledge, we combined different locomotion paradigms with pharmacology and knock-out of adrenergic receptors in mice expressing GCaMP6f specifically in astroglia. Our findings suggest different receptor involvements in global cortical astrocyte Ca^{2+} elevations during short bouts of arousal compared to states of sustained vigilance. This body of work can provide the basic groundwork to better understanding neural signaling in more complex behaviors involving neuromodulation.

Disclosures: **A.M. Salinas:** None. **M. Paukert:** None.

Poster

205. Mechanisms of Bi-Directional Glia-Neuron Communication

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Topic: B.11. Glial Mechanisms

Support: UK Multiple Sclerosis Society
Wellcome Trust-MRC Cambridge Stem Cell Institute
Daya Diri-Cambridge Trust Scholarship

Title: An integrated genomic approach to study astrocyte-induced maturation in human induced glutamatergic neurons

Authors: *M. KOTTER[1], M. ABDUL KARIM[1], K. BARANES[1], N. PATIKAS[2], E. METZAKOPIAN[2], S. COOPER[3], E. BELLO[3], A. BASSETT[3], D. TOURIGNY[4], J. O'NEIL[4]; [1]Clin. Neurosciences, [2]UK Dementia Res. Inst., Univ. of Cambridge, Cambridge, United Kingdom; [3]Wellcome Genome Campus, Wellcome Sanger Inst., Hinxton, United Kingdom; [4]MRC Lab. of Mol. Biol., Cambridge, United Kingdom

Abstract: Astrocytes are known to modulate many aspects of neuronal development, including their maturation, axon outgrowth and synapse formation. Whilst most studies of neurons are based on non-human models, the development of rapid neuronal reprogramming protocols has provided a unique opportunity to study the biology of human neurons. We have previously demonstrated that gene targeting the components of a genetic switch into genomic safe harbour sites enables optimised expression of NGN2 in hiPSC (OptiOx) and yields homogenous cultures of pure cortical glutamatergic neurons (hiNeuron) in less than four days. Here we aimed to gain a wholistic insight into how astrocytes modulate the function of human excitatory neurons. To assess electrophysiological activity, on the 3rdday of reprogramming, primary rat astrocytes were added to the hiNeuron cultures maintained on multi-array-electrodes (MEAs). Serial MEA recordings demonstrated spontaneous electrophysiological activity of hiNeurons as early as 11 days after induction. At 21 days post induction, synchronised burst patterns were detected across the network. In the absence of astrocytes hiNeurons cell bodies tended to cluster together and limited spontaneous electrophysiologic activity was recorded. The presence of astrocytes therefore profoundly affected neuronal function. In order to study genome-wide effects of astrocytes on hiNeurons, three independent biological samples were collected of hiNeurons cultured in the presence and absence of astrocytes at day 4, 14, and 21 following induction and submitted to bulk RNA-seq, bulk ATAC-seq, and single cell RNA-seq. Bulk sequencing data was de-convoluted according to species to separate human neuronal reads from those of rat astrocytes. The presence of astrocytes induced profound transcriptional and epigenetic changes in hiNeurons. Sc-RNA seq demonstrated that hiNeurons in the presence of astrocytes are distinct

from hiNeurons in the absence of glia.In conclusion, mixed-species *in vitro* cultures of astrocytes and hiNeurons are a useful tool to study human neuronal biology. The integration of RNA-Seq, ATAC-Seq, and scRNA-Seq provides unique and wholistic insights how the presence of astrocytes affects the function of human neurons.

Disclosures: **M. Kotter:** A. Employment/Salary (full or part-time):; Elpis Biomed. **M. Abdul Karim:** None. **K. Baranes:** None. **N. Patikas:** None. **E. Metzakopian:** None. **S. Cooper:** None. **E. Bello:** None. **A. Bassett:** None. **D. Tourigny:** None. **J. O'Neil:** None.

Poster

205. Mechanisms of Bi-Directional Glia-Neuron Communication

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 205.24/C42

Topic: B.11. Glial Mechanisms

Support: CIHR

Title: Contribution of astrocytic gap junctions and membrane potential to neocortical potassium redistribution

Authors: *A. EBRAHIM AMINI[1], P. BAZZIGALUPPI[2], B. STEFANOVIC[3], P. L. CARLEN[4];
[1]Biomed. and Biomaterial Engin., Toronto Western Hosp., Toronto, ON, Canada; [2]Med. Biophysics, Sunny Brook Hosp., Toronto, ON, Canada; [3]Imaging Res., Sunnybrook Hlth. Sci. Ctr., Toronto, ON, Canada; [4]Toronto Western Hosp, Toronto, ON, Canada

Abstract: Extracellular potassium ion concentration ([K]e) is tightly regulated throughout the brain and has a major impact on brain function. [K]e is significantly increased and plays a pathogenetic role in stroke, migraine and epilepsy. Increased [K]e causes neuronal depolarization and spreading depression. Astrocytes are thought to be key players in buffering [K]e since they express a high number of inwardly rectifying K channels, are very sensitive to changes in [K]e and are highly interconnected via gap junctions (GJs). GJs are cytoplasmic bridges allowing for intercellular transfer of molecules. [K]e redistribution in the neocortex is remarkably little studied. Herein I study [K]e redistribution using 2 novel techniques, focusing on the roles of astrocytic GJs and membrane potential. We hypothesize that: A) astrocytic GJ permeability strongly modulates [K]e redistribution in the neocortex, B) hyperpolarizing glia via optogenetics will depress resting [K]e and enhance [K]e reuptake via astrocytic membrane K rectifying channels. In this project we are using well-developed tools *in vivo* to elucidate role of astrocytes in neocortical K redistribution. Two double-barreled K-sensitive electrodes, each coupled with a local field potential (LFP) electrode, are placed about 3-4 mm apart into young adult CD-1 mouse neocortex. 50mM KCl solution is injected focally beside one of the K-LFP electrodes and

[K]e levels and LFP changes are measured; i) with application of GJ blockers or openers, and ii) with optical intervention, activating a hyperpolarizing glial construct introduced by a viral vector. We observed that focally increased [K]e is associated with a transient depolarization which spreads into the neighboring tissue. Topical application of the GJ blockers, carbenoxolone (non-specific) or Gap27 (Cx43 interastrocytic GJ blocker) to the exposed cortex increased the amplitude and duration of the [K]e and LFP responses to the raised [K]e in the proximal recording sites, whereas in the remote site, the [K]e and LFP responses were depressed and prolonged. Topical application of trimethylamine (a GJ opener) reduced the amplitudes of [K]e and LFP responses to the raised K both in the peri- and remote injection sites. Optical stimulation of the *in vivo* transfected neocortex decreased the response to the raised [K]e by about 35% both in the peri- and remote injection sites. In summary, these ongoing experiments suggest that interastrocytic gap junctional communication and astrocytic inward rectifying K channels importantly modulate extracellular [K]e in the neocortex.

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Poster

205. Mechanisms of Bi-Directional Glia-Neuron Communication

Location: Hall A

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Program #/Poster #: 205.25/C43

Topic: B.11. Glial Mechanisms

Support: CAPES Grant PDSE 88881.187392/2018-01

Title: Glucose regulates astrocyte-neuron signaling in the nucleus tractus solitarii

Authors: *C. MURAT[1], V. ARAQUE[2], R. LEÃO[1], P. KOFUJI[2];
[1]Univ. of São Paulo, Ribeirão Preto, Brazil; [2]Univ. of Minnesota, Minneapolis, MN

Abstract: Glucose sensing in the nucleus tractus solitarii (NTS), located in the dorsal medulla, participates in the regulation of feeding and blood glucose. Although several lines of evidence have demonstrated the modulatory role of glucose on neuronal NTS activity, little is known in regard to the role of glial cells in this process. Recent findings have established the bidirectional communication of neurons and astrocytes in diverse brain regions, but whether glucose affects the glial-neuronal signaling in the NTS remains unknown. Using acute NTS brain slices from mice, we have investigated whether extracellular glucose levels influence astrocyte calcium activity and gliotransmission. We have found that NTS astrocytes display spontaneous calcium elevations in the soma and processes, and that they responded to reductions of extracellular glucose (from 5 to 0.5 mM) by increasing the frequency of the calcium elevations. We also found the presence of spontaneous slow inward currents (SICs) in whole-cell recorded NTS

neurons under 5 mM glucose. These SICs displayed the hallmarks of excitatory events in neurons triggered by glutamate release from astrocytes, showing slower kinetics than excitatory postsynaptic currents (EPSCs), being unaffected by blocking action potential-mediated neuronal activity, and being sensitive to NMDA receptor blocker 2-amino-5-phosphonopentanoic acid (D-AP5). The SIC frequency was increased upon extracellular glucose reduction from 5 to 0.5 mM. Furthermore, the frequency increase of both calcium elevations and SICs evoked by extracellular glucose reduction were ablated in the IP_3R2 knockout mice, in which intracellular calcium mobilization is largely impaired in astrocytes. These results indicate the existence of astrocyte-neuron communication and gliotransmission in the NTS that can be regulated by extracellular glucose levels. Therefore, extracellular glucose changes can be sensed by NTS astrocytes, which then can influence neuronal excitability via glutamate release.

Disclosures: C. Murat: None. **V. Araque:** None. **R. Leão:** None. **P. Kofuji:** None.

Poster

205. Mechanisms of Bi-Directional Glia-Neuron Communication

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 205.26/C44

Topic: B.11. Glial Mechanisms

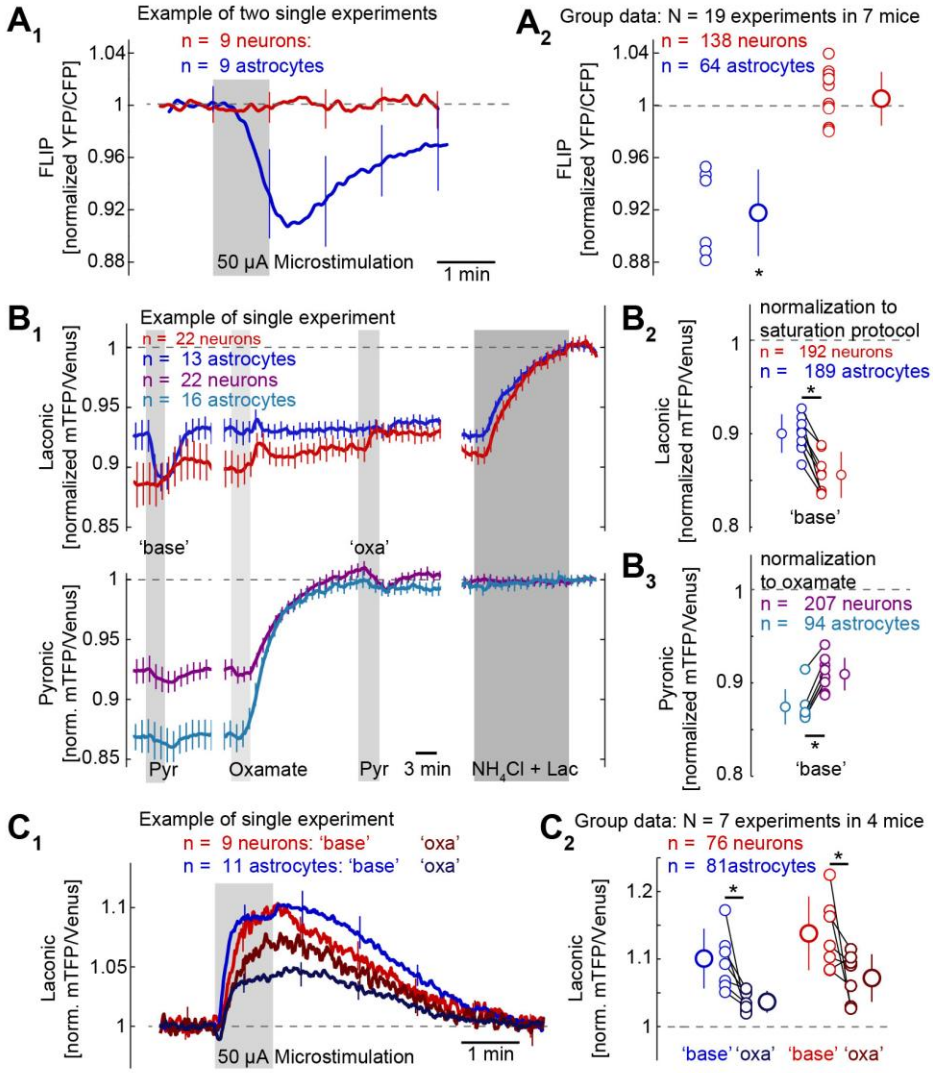
Support: Swiss National Science Foundation

Title: The pyruvate analogon oxamate reduces transacceleration and microstimulation-induced lactate transients in astrocytes and neurons in anesthetized mice

Authors: *P. MÄCHLER[1], M. T. WYSS[2], V. KAELIN[3], F. BARROS[4], B. WEBER[5];
[1]Neurosciences, UCSD, San Diego, CA; [2]Univ. of Zürich, Zürich, Switzerland; [3]Univ. of Zurich, Zurich, Switzerland; [4]Ctr. for Scientific Studies, Valdivia, Chile; [5]Inst. of Pharmacol. and Toxicology, Zurich, Switzerland

Abstract: Key players for the cellular compartmentation of brain energy metabolism are the monocarboxylate transporters (MCT), that facilitate the import and export of lactate, and the enzyme lactate dehydrogenase (LDH), that converts lactate into pyruvate and vice-versa. Oxamate is a substrate analogon to pyruvate and therefore interferes with LDH's and MCT's role in determining intracellular lactate levels. Therefore, we used the genetically encoded FRET sensors FLIIP (for glucose), Laconic (for lactate) and Pyronic (for pyruvate) in the anaesthetized mouse with two photon laser scanning microscopy.
1) While intravenous oxamate had no major effects on baseline Laconic signals, we found a sharp increase of Pyronic signals in astrocytes (12%) and neurons (9%), most likely due to a direct binding of oxamate to Pyronic (n=9). The temporal dynamics suggest a saturation of the Pyronic by oxamate in both cell types and therefore a higher baseline level of pyruvate in

neurons (4±1.2% higher, p<0.01).
2) The trans-acceleration driven export of lactate from astrocytes induced by intravenous pyruvate was reduced under oxamate, as indicated by a smaller drop of Laconic signals (1.7±1.6% smaller, p<0.01, n=9).
3) Intracortical electrical microstimulation with 50 µA pulse trains reduced astrocytic (8%, p<0.05) but not neuronal FLIIP, while Laconic increased in both cell types. The microstimulation-induced Laconic peak was reduced by oxamate in astrocytes (from 10.1±4.4% to 3.7±1.6%, p<0.01) and neurons (from 13.8±5.4% to 7.2±3.5%, p<0.05, n=7).



A) Intracortical electrical microstimulation with 50 µA reduced the astrocytic but not the neuronal glucose signal (FLIP). B) This difference was further investigated using intravenous application of the pyruvate substrate analogon oxamate, which induced saturation-like dynamics of the Pyronic signal, which indicates higher baseline pyruvate levels in neurons (B_3) while saturation of Laconic indicates higher lactate levels in astrocytes (B_2). C) Intravenous oxamate ('oxa') reduced the microstimulation induced lactate peak measured at baseline ('base') in astrocytes and neurons.

The microstimulation-induced decrease of glucose in astrocytes but not in neurons (3), the oxamate-induced interference with LDH- and MCT-activity in astrocytes and in neurons (2), and

the higher pyruvate levels in neurons as compared to astrocytes (1) are in agreement with a preference for astrocytic glycolysis and lactate export.

Disclosures: P. Mächler: None. **M.T. Wyss:** None. **V. Kaelin:** None. **F. Barros:** None. **B. Weber:** None.

Poster

205. Mechanisms of Bi-Directional Glia-Neuron Communication

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 205.27/C45

Topic: B.11. Glial Mechanisms

Support: CRG grant no. 2313 from King Abdullah University of Science and Technology
"KAUST-EPFL Alliance for Integrative Modeling of Brain Energy Metabolism
ETH Board funding to the Blue Brain Project

Title: Excitation states of metabolic networks predict ligand pulse phase signalling and dose-response fingerprinting

Authors: *J. S. COGGAN[1], D. KELLER[1], H. MARKRAM[1], F. SCHUERMANN[1], P. J. MAGISTRETTI[2];
[1]EPFL, Blue Brain Project, Geneva, Switzerland; [2]BESE, King Abdullah Univ. of Sci. and Technol., Thuwal, Saudi Arabia

Abstract: With a computational model of energy metabolism in an astrocyte, we analyze an enzymatic cascade, predict a novel intracellular signalling mechanism, and suggest a theoretical framework that could be employed in drug discovery or synthetic biology. Response trajectories of metabolites and enzymes between cAMP-stimulated glycogenolysis and the production of end-products such as lactate, ATP or NADH exhibit a host of non-linear dynamical response characteristics including hysteresis, response envelopes and dose-dependent phase transitions. We show how a system of enzymes can exist in multiple states, depending on the level of stimulation, and how the effects of ligands on this system will depend on those states. Indigenous or exogenous ligands may produce unique response "fingerprints" depending on the state of the system, a property that allows the creation of molecular switches to selectively tune the output. We conclude with the observation that dose-dependent phase transitions in our system, what we dub "ligand pulses" (LPs), resemble those of action potentials (APs) generated from excitatory postsynaptic potentials and suggest that both APs and LPs represent specialized cases of molecular phase signalling.

Disclosures: J.S. Coggan: None. **D. Keller:** None. **H. Markram:** None. **F. Schuermann:** None. **P.J. Magistretti:** None.

Poster

205. Mechanisms of Bi-Directional Glia-Neuron Communication

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 205.28/C46

Topic: B.11. Glial Mechanisms

Support: DARPA MTO HR-0011-16-1-0006

Title: Simulation of *in vivo* measurements of co-active neural, lactate, and glucose activity in a visual-cortical neuro-energetic network

Authors: R. NOACK[1], ***R. KOZMA**[2,3];
[1]CS, [2]Computer Sci., Univ. of Massachusetts Amherst, Amherst, MA; [3]Mathematics, Univ. of Memphis, Memphis, MA

Abstract: Aim: Construct a mathematical model and computer simulation of an *in vivo* study showing that extracellular changes in lactate and glucose concentrations closely correlate with spiking-neural activity during visual processing in a localized visual-cortical network.
Introduction: Currently, there is heated debate over just how the building blocks of cortical neuropil, the neuron-glia-vascular (NGV) assemblages, accomplish energy efficient information processing in the brain [1]. In this report we select an *in vivo* study [2] in the cat visual cortex that supports one of the leading hypotheses designed to model NGV dynamics, using the astrocyte-neuron lactate shuttle (ANLS) [2]. We build a mathematical model/computer of the NVG dynamics and explore its feasibility to interpret experimental findings.
Methods: The model we designed to simulate the ANLS is composed of four coupled first-order differential equations [3]. Two equations model the flow of energy through the NGV feedback loop, while another two equations describe how the spiking behavior of the neurons in the network is affected by the energy dynamics. Based on these equations, we develop a computer simulation of the spatio-temporal dynamics of the ANLS.
Results: The computer simulations produce behaviors, which closely match the results found in the target *in vivo* study [2]. High spiking activity of neurons in the network closely correlates with lactate and glucose concentrations, while the attenuation of neuron firing leads to return to baseline lactate and glucose activity. Furthermore, artificially lowering glucose and/or lactate concentrations in the simulation reversely influences the attenuation of neuron firing rates in an expected feedback fashion. Finally, high spiking activity of neurons in the network and associated lactate concentrations correlate with increased gamma-power rhythmic activity in the simulated network while low spiking activity/lactate concentration diminishes gamma activity.
Conclusion: In this report, we have constructed a mathematical model/computer simulation of the results found an ANLS-inspired *in vivo* study [2] in the cat visual cortex which closely matches those results and supports the ANLS hypothesis.

References:
1. Dienel, G. A. (2017) Journal of Neuroscience Research, 95: 2103-2125.
2. Li, B., Freeman, R. D. (2015) Journal of Neurochemistry, 135(4): 743-754.
3. Kozma, R., Noack, R., Manjesh, C. (2018) Proc. IEEE SMC2018: 722-727.

Disclosures: **R. Noack:** None. **R. Kozma:** None.

Poster

205. Mechanisms of Bi-Directional Glia-Neuron Communication

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 205.29/C47

Topic: B.11. Glial Mechanisms

Title: Glycogen functions in brain and muscle to maintain the free energy of rapid ATP hydrolysis

Authors: *R. A. SWANSON;
U.C.S.F., San Francisco, CA

Abstract: In brain, glycogen is stored primarily in astrocytes, whereas in muscle it is stored in the myocytes themselves. In both brain and muscle, glycogen is classically thought to serve as a local supply of glucose. However, experimental data do not support any physiological condition under which glucose supply to either brain or muscle becomes rate limiting for energy production. Glycogen is nevertheless utilized continuously and required for optimal function of muscle (and brain), even when free glucose is available, and despite a net energetic cost of shuttling glucose on and off glycogen polymer. The purpose of this apparently wasteful shunt can be explained by a thermodynamic consideration of how glycogen metabolism effects the amount of energy derived from ATP hydrolysis. The capacity of cells to perform energy - dependent processes is determined not only by ATP availability but also by the amount of energy that is obtained from ATP hydrolysis (ATP --> ADP + Pi). This energy, the $\Delta G'_{ATP}$, declines as ADP and Pi levels rise at the sites of ATP hydrolysis. At sites were ATP is hydrolyzed faster than it is regenerated, the resulting rise in ADP and Pi reduce the amount of energy obtained from ATP. This effect is countered in part by the creatine phosphokinase reaction, which buffers the local rise in ADP levels (PCr + ADP --> ATP + Cr). However, the phosphate initially bound in PCr is released as free Pi as the ATP is hydrolyzed. Crucially, this rise in Pi can be buffered by the glycogen phosphorylase reaction ($glycogen_n$ + Pi --> $glycogen_{n-1}$ + glucose-1-phosphate). The buffering effect of glycogen on Pi elevations can be estimated to increase $\Delta G'_{ATP}$ by up to 13 % at the point of cellular PCr depletion. This thermodynamic function of glycogen provides an explanation for the its requirement during rapid energy demand. It also provides an explanation for glycolytic super-compensation when glycogen is not available, for the co-

localization of glycogen and cytosolic PCr in brain astrocytes, and for aspects of exercise physiology in muscle glycogen phosphorylase deficiency (McArdle's disease).

Disclosures: R.A. Swanson: None.

Poster

206. Molecular and Cellular Mechanisms of Demyelinating Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 206.01/C48

Topic: B.12. Demyelinating Disorders

Support: NMSS Postdoctoral Fellowship: FG-1607-25381
Mayo Clinic Center for MS and Autoimmune Neurology Pilot Award
Global MS Research Booster Award 17-997 MS (Dutch MS Research Foundation)

Title: Demyelination and interferon signaling in axons cause retrograde upregulation of neuronal ISGylation pathway genes

Authors: *B. CLARKSON, C. L. HOWE;
Neurol., Mayo Clin., Rochester, MN

Abstract: Cognitive impairment in MS is associated with diffuse gray matter injury, which remains poorly understood. Given that axons span both white and gray matter regions, it's possible that signals within these axons contribute to the spread of diffuse injury in the brain. We show that retrograde IFNg signaling in axons (in vitro human and murine) and demyelination (murine in vivo) cause transcriptional / translational changes in neuronal cell bodies. Chief among the candidate signaling pathways identified is ISGylation, a process whereby a multitude of cellular proteins are modified by the attachment of one or more ISG15 molecules. Using custom adeno-associated viral vectors we tested how altering ISGylation in neurons affects neuronal synaptic function and how it affects neuronal responsiveness to specific inflammatory factors in cell cultures. We also measured ISGylation in MS brain tissues to see if neuronal ISGylation correlates with gray matter injury in MS. We report early evidence that increased neuron ISGylation alters the composition of neuron-derived extracellular vesicles. Microglia treated with extracellular vesicles from neurons overexpressing ISGylation pathway genes exhibited morphology consistent with increased phagocytic activity and increased mRNA expression of inflammatory cytokines including: CCL2, IL1b, TNFa, iNOS, CXCL10, CCL5, and IL6. At this point, it is still unclear how these changes are related to gray matter pathology. However, it is possible that microglial activation by these vesicles is involved with driving synapse loss as activated microglia are known to be capable of stripping neuronal synapses.

Disclosures: **B. Clarkson:** None. **C.L. Howe:** None.

Poster

206. Molecular and Cellular Mechanisms of Demyelinating Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 206.02/C49

Topic: B.12. Demyelinating Disorders

Support: NIH Grant NS094151

Title: Oligodendrocyte-specific NF-κB activation protects mice against experimental autoimmune encephalomyelitis

Authors: ***W. LIN**[1], Y. YUE[2];
[1]Univ. of Minnesota Dept. of Neurosci., Minneapolis, MN; [2]Univ. of Minnesota, Minneapolis, MN

Abstract: The transcription factor NF-κB plays a critical role in inflammatory diseases by regulating inflammation and cell viability. Activation of NF-κB is observed in oligodendrocytes in multiple sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE). Our previous *in vitro* and *in vivo* studies showed that inactivation of NF-κB in oligodendrocytes via enforced expression of IκBαΔN (a super-suppressor of NF-κB) increases their sensitivity to the cytotoxicity of inflammatory mediators. In this study, we sought to determine the effects of enhanced activation of NF-κB on oligodendrocytes during EAE using a mouse model that expresses constitutively active form of IκB kinase 2 (IKK2ca) specifically in oligodendrocytes. We demonstrated that expression of IKK2ca led to NF-κB activation in oligodendrocytes, but did not affect their viability or function under normal condition. Importantly, we found that enhanced activation of NF-κB specifically in oligodendrocytes significantly attenuated EAE disease severity and ameliorated EAE-induced oligodendrocyte loss, demyelination, and axon degeneration. Conversely, enhanced activation of NF-κB specifically in oligodendrocytes did not alter inflammation in EAE mice. Our results demonstrate that NF-κB activation in oligodendrocytes is cytoprotective, protecting mice against EAE.

Disclosures: **W. Lin:** None. **Y. Yue:** None.

Poster

206. Molecular and Cellular Mechanisms of Demyelinating Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 206.03/C50

Topic: B.12. Demyelinating Disorders

Support: NMSS P65725

Title: Activation of the necroptosis signaling cascade in cortical neurons in MS

Authors: *C. PICON MUNOZ, R. JAMES, N. D. MAZARAKIS, R. REYNOLDS;
Imperial Col. London, London, United Kingdom

Abstract: Increased cortical pathology in secondary progressive MS (SPMS) is associated with more a severe clinical course and the presence of subpial grey matter (GM) lesions with significant neuronal loss and inflammatory infiltrates in the subarachnoid space. Our previous work suggests that TNF could be a key molecule driving this cortical pathology. We investigated changes in the balance in the TNF signaling pathway in GM tissue blocks from 30 SPMS post-mortem brains and 10 controls using immunocytochemistry and Western blotting. TNFR1 was significantly up-regulated in SPMS compared to controls, while no differences were found in the expression levels of TNFR2. A downregulation of CYLD and up-regulation of FLIP-L was found in SPMS compared to controls, key proteins involved in the regulation of TNF pathway towards cell death. MS cases showed a down-regulation of the cleaved active form of caspase 8 and no differences were found in the number of cleaved caspase 3 cellsTUNEL positive cells between groups. In contrast, MS cases showed a significant increase in the key proteins of the necroptotic pathway, phospho-RIPK3 and phospho-MLKL (p-MLKL), which localized predominantly to neurons. The density of neurons expressing p-MLKL and p-RIP3 was significantly increased in MS cases compared to controls. We found MLKL oligomers only in MS, a sign of activated necroptosis. We injected lentiviral vectors carrying the TNF and interferon-gamma genes into the subarachnoid space of DA rats. Persistent cytokine production over 1 month in DA rats produced chronic meningeal inflammation and increased levels of the necroptosis markers RIPK1, p-MLKL and p-RIPK3 in the underlying cortical neurons. Taken together our data show that in SPMS there is a shift in the balance of TNF dependent signaling pathways towards TNFR1-mediated necroptosis in cortical neurons, which could be responsible for the neurodegeneration observed in the GM of MS patients.

Disclosures: C. Picon Munoz: None. **R. James:** None. **N.D. Mazarakis:** None. **R. Reynolds:** None.

Poster

206. Molecular and Cellular Mechanisms of Demyelinating Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 206.04/C51

Topic: B.12. Demyelinating Disorders

Support: Canadian Institutes of Health Research
MS Society of Canada

Title: Role of ferroptosis in experimental autoimmune encephalomyelitis and cuprizone-induced demyelination

Authors: *P. JHELUM, E. SANTOS-NOGUEIRA, A. HAUMONT, S. DAVID;
McGill Univ. Hlth. Ctr., Montreal, QC, Canada

Abstract: A new regulated form of non-apoptotic cell death called “ferroptosis” was identified in cancer and recently in neurodegenerative disease. Ferroptosis is an iron-dependent form of cell death which requires iron mediated free radical generation that triggers lipid peroxidation in the absence of sufficient amounts of glutathione mediated protection. Oxidative damage and iron toxicity are thought to contribute to the pathogenesis of Multiple Sclerosis (MS) and Experimental autoimmune encephalomyelitis (EAE), an animal model used to study MS. We therefore assessed if ferroptosis plays a role in EAE and in the cuprizone model of demyelination. We assessed the mRNA expression of several ferroptosis markers involved in glutathione metabolism and cellular iron cycling in models of relapsing-remitting EAE (RR-EAE) and chronic EAE (CH-EAE). Expression of ferroptosis markers such as ACSF2, ACSL4, and COX-2 are greater in CH-EAE as compared to RR-EAE. Ferroptosis markers related to cellular iron cycling, IREB2, TfR1 and NCOA4 are also increased in CH-EAE but not RR-EAE. This suggests mobilization of ferritin to the autophagosome (via NCOA4) leading to release of bioavailable iron, and increased iron uptake (via TfR1), which generate iron-mediated free radicals that trigger lipid peroxidation. Importantly, our preliminary studies show that ferrostatin-1 (inhibitor of ferroptosis) improves outcome in CH-EAE when treatment is started at the time of onset of symptoms. These results provide evidence that ferroptosis is likely to occur in CH-EAE. In addition, we also studied role of ferroptosis in cuprizone-induced demyelination in mice. Cuprizone is a copper chelator that induces oligodendrocyte (OL) cell death and demyelination in the CNS, which is particularly seen in the corpus callosum (CC). As copper-containing enzymes play a role in iron homeostasis and controlling oxidative stress, we assessed if copper chelation by CZ leads to iron-mediated toxicity and results in OL loss. We found that there is ~ 65% loss of mature OL in the CC as early as 2 days after start of CZ and 80% loss after 1 week. This early OL cell death is accompanied with the expression of several markers of ferroptosis. Furthermore, treatment with ferrostatin-1 prevents this early CZ-induced OL loss at 2 days and

reduces myelin loss at 4 weeks, suggesting that loss of copper may contribute to iron-mediated OL death and demyelination by ferroptosis.

Disclosures: P. Jhelum: None. **E. Santos-Nogueira:** None. **A. Haumont:** None. **S. David:** None.

Poster

206. Molecular and Cellular Mechanisms of Demyelinating Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 206.05/C52

Topic: B.12. Demyelinating Disorders

Support: NIH Grant ZIAHD000713-22

Title: A mouse model with deletion of the thrombin cleavage site in Neurofascin 155

Authors: *R. D. FIELDS[1], D. J. DUTTA[2];
[1]Section on Nervous Syst. Develop. and Plasticity, [2]Natl. Inst. of Child Hlth. and Human Develop., NIH, Bethesda, MD

Abstract: The myelin sheath is attached to the paranodal region flanking the node of Ranvier as uncompacted paranodal loops via septate cell-adhesion junctions where glial Neurofascin 155 (NF155) interacts with axonal Caspr1-Contactin1 complex. Thrombin in the CNS can cleave Neurofascin 155 at AA924-926 to disrupt this interaction resulting in detachment of paranodal loops of myelin from the axon and consequent thinning of the myelin sheath. Perinodal astrocytes inhibits this process by engulfing the node and releasing thrombin inhibitors. This remodeling of the myelin sheath has implications for both CNS plasticity and pathology. Unregulated thrombin-mediated cleavage of NF155 due to changes in perinodal astrocyte morphology, astrocyte secretome and influx of vascular thrombin via a compromised blood-brain barrier (BBB) can be antigenic to NF155 and its interacting partners. Consistent with this notion, the node of Ranvier around compromised BBB in normal appearing white matter is among the earliest loci of pathology in MS. Autoantibodies to Caspr1, Neurofascin 155 and Contactin1 are prevalent in various inflammatory demyelinating neuropathies of the nervous system including multiple sclerosis. Specifically, autoantibodies to NF155 has been mapped to the third Fibronectin IIII domain of NF155, which also houses the thrombin cleavage site. We deleted the nucleotides corresponding to the thrombin cleavage site of NF155, AA924-926, in mice using Crispr-Cas9 gene editing. Dysmyelination is evident with widespread eversion of paranodal loops of myelin. The nodal gap is significantly enlarged. There is a complete loss of paranodal septate junctions. Paranodal Caspr1-Contactin1, nodal Na+ channels and juxtaparanodal K+ channels are mislocalized. Young adult mice have severe tremors and ataxia. Biochemical and mass-spectrometric analysis shows no evidence for the NF155 protein, however other members

of the Neurofascin family are present. This is surprising considering deletion of another larger domain in NF155, Immunoglobulin 5-6, results in a truncated protein that is expressed normally. Thus, deletion of the thrombin cleavage site in NF155 phenocopies deletion of the NF155 protein, indicating that the thrombin cleavage site in NF155 is essential for its structural integrity and its deletion results in a misformed protein targeted for proteolytic degradation upon translation.

Disclosures: R.D. Fields: None. **D.J. Dutta:** None.

Poster

206. Molecular and Cellular Mechanisms of Demyelinating Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 206.06/C53

Topic: B.12. Demyelinating Disorders

Support: JSPS KAKENHI Grant JP16K09982
JSPS KAKENHI Grant JP19K08270

Title: Impairment of small RNAs into mitochondria can cause abnormal myelination and neuronal cell death

Authors: *M. UEMATSU, Y. NUMATA-UEMATSU, A. KIKUCHI, R. SATO, N. ARAI-ICHINOI, S. KURE;
Pediatrics, Tohoku Univ. Grad. Sch. of Med., Sendai, Japan

Abstract: <Background>Recent studies suggest that impaired transcription or mitochondrial translation of small RNAs can cause abnormal myelination. However, the mechanism is still unclear. We previously reported 2 siblings with PNPT1 mutations who presented delayed myelination. A polynucleotide phosphorylase (PNPase) encoded by PNPT1 facilitates the import of small RNAs into mitochondria. <Method> To analyze the relationship between the dysfunction of small RNA import into mitochondria and myelin abnormalities, we performed a mitochondrial RNA processing assay in patient skin fibroblasts, because PNPase is required for the processing of mitochondrial RNA transcripts. In addition, to analyze neuronal cell function, we performed direct conversion of the patient's derived fibroblasts to neurons by reprogramming PTB-regulated microRNA. In vitro myelination experiments using dorsal root ganglia (DRG) explant, knockdown of PNPT1 and POLR3B by siRNA was examined to investigate immunohistological analysis of peripheral neuron.<Results> Analyses of fibroblasts from the patient showed that PNPase expression was markedly decreased and that import of the small RNA RNase P into mitochondria was impaired. Exogenous expression of wild-type PNPT1, but not mutants, rescued ATP production in patient skin fibroblasts, suggesting the pathogenicity of the identified mutations. Using direct conversion, the patient's nerve cells were differentiated

from the patient's fibroblasts. We found the converted patient’s nerve cells developed apoptosis in the early period. Peripheral neuromyelination culture using DRG showed dysfunction of PNPT1 cause impairment of axonal growth and myelination. <Conclusion> Our cases with neurodevelopmental diseases with mitochondrial dysfunction expand the phenotypic spectrum of PNPT1 mutations that can cause delayed myelination. This study suggests impairment of small RNAs into mitochondria can cause abnormal myelination and neuronal cell death.

Disclosures: **M. Uematsu:** None. **Y. Numata-Uematsu:** None. **A. Kikuchi:** None. **R. Sato:** None. **N. Arai-Ichinoi:** None. **S. Kure:** None.

Poster

206. Molecular and Cellular Mechanisms of Demyelinating Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 206.07/C54

Topic: B.12. Demyelinating Disorders

Support: NIH Grant R01 NS100510

Title: Epigenomic regulation of nerve injury genes in Schwann cells

Authors: P. DUONG[1], K. MAH[1], ***R. RAMESH**[1], R. AWATRAMANI[2], J. SVAREN[1]; [1]Univ. of Wisconsin Madison, Madison, WI; [2]Northwestern Univ., Chicago, IL

Abstract: After peripheral nerve injury, terminally differentiated Schwann cells undergo a dramatic transformation to a proliferative and regenerative state that supports the ultimate regeneration of axons. While many transcriptional regulators of this nerve injury transition have been identified, reprogramming of Schwann cells to a repair state requires manipulation of epigenomic pathways. We have previously shown that many nerve repair genes are regulated by the repressive histone H3K27 trimethylation (H3K27me3) mark, which is deposited by Polycomb Repressive Complex 2 (PRC2). For example, H3K27me3 is depleted from nerve repair genes such as sonic hedgehog and glial-derived neurotrophic factor (Shh and Gdnf) after injury. These two genes are required for the pro-regenerative activities of Schwann cells. Therefore, our hypothesis is that removal of polycomb repression is required for nerve injury genes. To determine how polycomb repression could be reversed after injury, we have developed Schwann cell specific double knockouts of H3K27 demethylases JMJD3/KDM6B and UTX/KDM6A. A morphological analysis of the knockout mice was performed in a blinded manner, employing males and females. Myelin formation is unaltered in the absence of H3K27 demethylase activity, but analysis of nerve injury in these knockout lines show that the early induction of some injury genes is dependent on H3K27 demethylation. In addition, repression by H3K27me3 is also genomically colocalized with deposition of H2A monoubiquitination by Polycomb Repressive Complex 1. Lastly, we found that a Schwann cell knockout of microRNA

function via deletion of Dgcr8 induced a similar set of genes to nerve injury genes that are derepressed in the absence of PRC2. Therefore, our studies have identified a mechanistic link between microRNA's and polycomb regulation of nerve injury genes in Schwann cells.

Disclosures: P. Duong: None. **K. Mah:** None. **R. Ramesh:** None. **R. Awatramani:** None. **J. Svaren:** None.

Poster

206. Molecular and Cellular Mechanisms of Demyelinating Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 206.08/C55

Topic: B.12. Demyelinating Disorders

Support: NIH Grant NS098170 (JC and CS)
NeuroCures Foundation, Inc. (JC)

Title: Dynamic response of microglia/macrophage polarization following demyelination in mice

Authors: T. CHU[1], Y. ZHANG[4], Z. TIAN[5], C. YE[6], M. ZHU[2], L. B. E. SHIELDS[4], G. N. BARNES[3], C. B. SHIELDS[4], *J. CAI[1];
[1]Pediatrics, [2]Radiology, [3]Neurol., Univ. of Louisville Sch. of Med., Louisville, KY; [4]Norton Neurosci. Institute, Norton Healthcare, Louisville, KY; [5]Orthopedics, China-Japan Union Hosp. of Jilin Univ., Changchun, China; [6]Pediatrics, The Second Affiliated Hosp. and Yuying Children's Hosp. of Wenzhou Med. Univ., Wenzhou, China

Abstract: Background: The glial response in multiple sclerosis (MS), especially for recruitment and differentiation of oligodendrocyte progenitor cells (OPCs), predicts the success of remyelination of MS plaques and return of function. As a central player in neuroinflammation, activation and polarization of microglia/macrophages (M/M) that modulate the inflammatory niche and cytokine components in demyelination lesions may impact the OPC response and progression of demyelination and remyelination. However, the dynamic behaviors of M/M and OPCs during demyelination and spontaneous remyelination are poorly understood, and the complex role of neuroinflammation in the demyelination-remyelination process is not well known. In this study, we utilized two focal demyelination models with different dynamic patterns of M/M to investigate the correlation between M/M polarization and the demyelination-remyelination process.
Methods: The temporal and spatial features of M/M activation/polarization and OPC response in two focal demyelination models induced by lysolecithin (LPC) and lipopolysaccharide (LPS) were examined in mice. Detailed discrimination of morphology, sensoriomotor function, diffusion tensor imaging (DTI), inflammation-relevant cytokines, and glial responses between these two models were analyzed at different phases.

Results: The results show that LPC and LPS induced distinctive temporal and spatial lesion patterns. LPS produced diffuse demyelination lesions, with a delayed peak demyelination and functional decline compared to LPC. Oligodendrocytes, astrocytes, and M/M were scattered throughout the LPS-induced demyelination lesions but were distributed in a layer-like pattern throughout the LPC-induced lesion. The specific M/M polarization was tightly correlated to the lesion pattern associated with balance beam function.
Conculsions: This study elaborated on the spatial and temporal features of neuroinflammtion mediators and glial response during the demyelination-remyelination processes in two focal demyelination models. Specific M/M polarization is highly correlated to the demyelination-remyelination process probably via modulations of the inflammatory niche, cytokine components, and OPC response. These findings not only provide a basis for understanding the complex and dynamic glial phenotypes and behaviors, but also reveal potential targets to promote/inhibit certain M/M phenotypes at the right timing for efficient remyelination.

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Poster

206. Molecular and Cellular Mechanisms of Demyelinating Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 206.09/C56

Topic: B.12. Demyelinating Disorders

Title: Subarachnoid hemorrhage results in hippocampal atrophy and suppression of oligodendrocyte genes expression

Authors: *A. S. REGNIER-GOLANOV, M. S. HERNANDEZ, C. KARMONIK, L. M. PHAN, L. E. PETERSON, E. V. GOLANOV, G. W. BRITZ;
Neurosurg., Houston Methodist Hosp., Houston, TX

Abstract: Forty five percent of subarachnoid hemorrhage (SAH) survivors are unable to continue with their activities due to permanent cognitive or emotional disabilities accompanied by atrophy of temporomesial area. The mechanism of post-SAH hippocampal atrophy remains unclear. We explored changes in hippocampal myelination, volume and expression of oligodendrocyte-related genes expression and long-term behavioral abnormalities following the filament-induced SAH in mice. We used RNA sequencing to explore the changes in gene expression 4-days after SAH. Under deep anesthesia animals were decapitated and hippocampi extracted. Hippocampal RNA of the SAH (n=4) and sham (no perforation, n=3) groups was extracted. Differential gene expression analysis was performed (-1.5<fold change>1.5; $p<0.05$). For imaging and immunohistochemistry (IHC), deeply anesthetized animals were perfused with saline followed by 4% PFA; brains were extracted. T2 weighted images were acquired using a

TurboRARE_3D MRI sequence of the brains in agar filled tubes placed in a bore-mounted coil (Bruker). Hippocampal volume was manually measured using ITK-SNAP. Brain cryo-slices were processed for IHC, and fluorescence was quantitatively assessed. Nissl staining, FJC or caspase 3 staining did not reveal neuronal cell loss in CA1 or DG areas. Gene set enrichment analysis of all genes revealed a significant (FDR <0.15) reduction of 32 oligodendrocyte's genes expression in CAHOY set, indicating decrease in oligodendrocytic phenotype cells. It was accompanied by decrease of immunofluorescence of myelin staining (by 33%) and its level (by WB) in hippocampi (by 30%, $p<0.05$) of SAH animals. Hippocampal volume as measured by MRI in mice 4 d after SAH (22.7 ± 1.2 mm^3) was significantly (n=3/group, $p<0.001$) diminished compared to sham (26.3 ± 0.47 mm^3). Behavior analysis demonstrated long-term (up to 8 months) abnormalities in SAH vs. sham animals. Starting at 30 d after the surgery and persistently (up to 8 months) SAH animals demonstrated significantly higher anxiety (15%, $p<0.005$, n=8-10; and 12%, $p<0.04$, n=6-8, resp.; open field test) than sham animals. Persisting higher level of anxiety in SAH animals was confirmed by light-dark test (tendency increased by 16%, $p<0.088$, n=6-8). Spatial memory abnormalities were divulged by Y-maze test, by the observations that SAH animals showed tendency to choose the arm 10% ($p=0.088$, n=10-12) more randomly and that at 30 days SAH animals used direct escape search strategy 22% ($p<0.05$, n=8-10) less than sham animals. Our data demonstrate that SAH induces hippocampal atrophy, which is comparable to such in humans, and accompanied by homologous behavioral changes.

Disclosures: A.S. Regnier-Golanov: None. **M.S. Hernandez:** None. **C. Karmonik:** None. **L.M. Phan:** None. **L.E. Peterson:** None. **E.V. Golanov:** None. **G.W. Britz:** None.

Poster

206. Molecular and Cellular Mechanisms of Demyelinating Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 206.10/C57

Topic: B.12. Demyelinating Disorders

Title: Metabolic manipulation of immune partners in central nervous system inflammation shapes cytotoxic T cell activation

Authors: *E. M. GRUND[1], M. M. STANDIFORD[2], B. D. S. CLARKSON[3], C. L. HOWE[4]; [1]Mayo Clin. Med. Scientist Training Program, Rochester, MN; [2]Mayo Grad. Sch., Rochester, MN; [4]Neurol., [3]Mayo Clin., Rochester, MN

Abstract: The progressive phase of Multiple Sclerosis (MS) is understood to involve axonal injury in the presence of activated microglia and clonally-expanded CD8 T cells. Preclinical models of central nervous system (CNS) demyelination have demonstrated the importance of local antigen presentation in re-stimulating infiltrating peripheral lymphocytes. Antigen presentation by resident microglia or infiltrating dendritic cells is impacted by ongoing

phagocytosis of myelin debris. Furthermore, evidence suggests that the activation status and effector functions of both microglia and T cells are modulated by the metabolites available within a demyelinated, inflammatory milieu. We show that microglia are competent to present the immunodominant H2Kb-restricted epitope (SIINFEKL) from the prototypical neoantigen ovalbumin and that CD8 T cells specific for this antigen (OT-1) proliferate and upregulate canonical activation markers following co-culture. We next determined the metabolic pathways engaged by microglia and T cells upon exposure to antigen and how these pathways were altered by the presence of myelin or neuron debris. Finally, we studied how modulating these metabolic pathways by manipulating lipid and nucleotide availability alters microglial and T cell activation profiles. Understanding the metabolic determination of effector functions within these populations during demyelination is important to mechanistically appreciate the action of current MS therapies such as teriflunomide during disease progression. An appreciation of the metabolic control of effector functions in the CNS may provide possible new therapeutic strategies for minimizing progression in autoimmune diseases such as MS.

Disclosures: E.M. Grund: None. **M.M. Standiford:** None. **B.D.S. Clarkson:** None. **C.L. Howe:** None.

Poster

206. Molecular and Cellular Mechanisms of Demyelinating Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 206.11/C58

Topic: B.12. Demyelinating Disorders

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Title: Cnp-Cre;Mek1DD/+ mice exhibit attenuated experimental autoimmune encephalomyelitis and increased regulatory B10 cell activation

Authors: *M. A. JEFFRIES[1], A. E. OBR[1], K. URBANEK[2], S. L. FYFFE-MARICICH[2], T. L. WOOD[1];
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Abstract: In demyelinating diseases such as multiple sclerosis (MS), loss of myelin and oligodendrocytes (OLs) in the central nervous system (CNS) results in progressive decline in function. Current therapies do not adequately prevent demyelination by suppressing immune function or promoting OL resistance and myelin repair. Therefore, additional research to elucidate mechanisms of limiting demyelination is critical to increase therapeutic efficacy.

Previous research has revealed that sustained ERK1/2 activation in the OL lineage results hypermyelination. We initially sought to determine whether increased ERK1/2 signaling in the OL lineage 1) protects against experimental autoimmune encephalomyelitis (EAE) induction or 2) promotes enhanced remyelination and recovery after induction of EAE. In initial experiments, we observed that constitutive activation of ERK1/2 in *Cnp-Cre;Mek1DD-eGFP/+* mice, which express MEK1DD and GFP under the *Cnp* promoter in OLs, resulted in a significant decrease in EAE clinical severity compared to controls. To determine whether MEK1DD expression in the OL lineage was protective or reparative, we used tamoxifen-inducible *Plp-Cre^{ERT};Mek1DD-eGFP/+* mice that express MEK1DD in mature OLs. *Plp-Cre^{ERT};Mek1DD-eGFP/+* mice given tamoxifen 40 days prior to EAE induction did not exhibit altered disease course, indicating sustained ERK1/2 activation in mature OLs does not protect against EAE. When given tamoxifen at first symptom, EAE clinical score in *Plp-Cre^{ERT};Mek1DD-eGFP/+* mice did not improve, also suggesting no reparative effect. However, when examining immune cells we found that the *Cnp* promoter, thought to be OL lineage-specific, resulted in GFP+ recombined CD19+ B-cells and CD3+ T-cells. While ERK1/2 signaling in T-cells is known to result in activation of EAE-inducing Th17 cells and suppression of regulatory T-cells, no studies have examined the role of ERK1/2 signaling in regulatory B-cells. Using flow cytometry, we determined that the initial regulatory B10 cell population did not differ in control and *Cnp-Cre;Mek1DD-eGFP/+* spleens. However, after *in vitro* stimulation with lipopolysaccharide, recombined B-cells exhibited a 5-fold increase in the number of B10 cells compared to controls. These data are important as previous studies showed that B10 cells can suppress the initiation of EAE in a manner consistent with that observed in *Cnp-Cre;Mek1DD-eGFP/+* mice. Taken together, our data support the conclusion that sustained ERK1/2 activation in B-cells suppresses immune-mediated demyelination via increasing activation of regulatory B10 cells.

Disclosures: **M.A. Jeffries:** None. **A.E. Obr:** None. **K. Urbanek:** None. **S.L. Fyffe-Maricich:** None. **T.L. Wood:** None.

Poster

206. Molecular and Cellular Mechanisms of Demyelinating Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 206.12/C59

Topic: B.12. Demyelinating Disorders

Support: NeuroCures Foundation, Inc. (JC)
National Natural Science Foundation of China (YW)
Jilin Provincial Key Laboratory of Bone and Joint Degenerative Diseases (QZ)

Title: Platelet activating factor deteriorates lysophosphatidylcholine-induced demyelination via its receptor-dependent and -independent mechanisms

Authors: Z. TIAN[1,2], *T. CHU[2], L. B. E. SHIELDS[4], Q. ZHU[1], Y. ZHANG[4], G. N. BARNES[3], Y. WANG[5], C. B. SHIELDS[4], J. CAI[2];
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Abstract: Accumulating evidence suggests that platelet activating factor (PAF) increases the inflammatory response in demyelinating diseases such as multiple sclerosis. However, PAF receptor (PAFR) antagonists did not show therapeutic efficacy for MS and its underlying mechanisms remain poorly understood. In the present study, we investigated the effects of PAF on an *ex vivo* demyelination cerebellar model following lysophosphatidylcholine (LPC, 0.5 mg/ml) application using wild-type and *PAFR* conventional knockout (*PAFR*-KO) mice. Demyelination was induced in cerebellar slices that were cultured with LPC for 18 hours. Exogenous PAF (1 μM) acting on cerebellar slices alone did not cause demyelination but increased the severity of LPC-induced demyelination in both wild-type and *PAFR*-KO mice. LPC inhibited the expression of PAF-AH, MBP, TNF-α and TGF-β1 but facilitated the expression of IL-1β and IL-6 in wild-type preparations. The inflammatory cytokine expression was modulated following exogenous PAF application with or without LPC that included the pro-inflammatory cytokines TNF-α, IL-1β and IL-6, and the anti-inflammatory cytokine TGF-β1. The syntheses of IL-1β and IL-6 were significantly increased in LPC-induced demyelination preparations without PAF but showed a redundancy in PAF-treated wild-type and mutant slices, suggesting that PAF plays a detrimental role in LPC-induced demyelination via PAFR-dependent and PAFR-independent pathways.

Disclosures: Z. Tian: None. **T. Chu:** None. **L.B.E. Shields:** None. **Q. Zhu:** None. **Y. Zhang:** None. **G.N. Barnes:** None. **Y. Wang:** None. **C.B. Shields:** None. **J. Cai:** None.

Poster

206. Molecular and Cellular Mechanisms of Demyelinating Disorders

Location: Hall A

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Program #/Poster #: 206.13/C60

Topic: B.12. Demyelinating Disorders

Support: NIH R21NS093487
National Multiple Sclerosis Society RG1703

Title: Axonal damage and behavioral correlates in myelin repair after chronic CNS demyelination

Authors: *G. SAMTANI[1], D. MICHAUD[2], K. KONGANTI[3], S. KIM[2], J. LI[1];
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Abstract: The proper function of the vertebrate central nervous system (CNS) is critically dependent on myelination of axon fibers. Loss of myelin or myelin-producing oligodendrocytes (OLs) contributes to neurological disorders such as multiple sclerosis and Alzheimer's disease as well as to age-related neurological decline. It is generally believed that the remyelination of denuded axon fibers confers protection. However, our understanding of the myelin repair process, specifically the interaction between axons, myelin, and glial cells, remains limited.
In this study, we utilized the cuprizone (CPZ) model to investigate cellular interactions during myelin repair. To facilitate direct visualization of myelin, we utilized "green-myelin" mice that express membrane-anchored green fluorescence protein in mature OLs under the *CNP1* promoter. Adult mice were fed a 12-week "chronic" CPZ diet, which induced substantial demyelination and glial activation in the corpus callosum (CC) and cortex (Ctx). After 4-8 weeks of recovery, significant remyelination and decreased microgliosis were achieved, but astrogliosis was maintained. Further, staining of amyloid precursor protein and non-phosphorylated Neurofilament H revealed significant axonal damage in both the CC and Ctx, some of which persisted even after 8 weeks of recovery. LC3-II^{+} vesicles were observed in microglia and, interestingly also appeared to be in axons, suggesting aberrant autophagy activities in these axons.
DigiGait and Open Field behavioral analyses demonstrated a worsening of bilateral sensorimotor function after chronic demyelination, indicated by a shorter, shuffling stride, decreased distance traveled, and prolonged postural stability adjustments, which was not rescued after myelin recovery. Transcriptome profiling at various stages of demyelination and myelin repair revealed common and distinct pathways for myelin repair at early and chronic stages. Together, our data suggest that persistent axonal damage due to a chronic demyelinating insult may underlie aberrant remyelination mechanisms and prolonged motor deficits in demyelinating disorders.

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Poster

206. Molecular and Cellular Mechanisms of Demyelinating Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 206.14/C61

Topic: B.12. Demyelinating Disorders

Support: Abbott labs

Title: Quantifying myelination of single neurons using spatial light interference microscopy (SLIM)

Authors: ***Y. LEE**[1,3], M. FANOUS[1,4], A. LOUIE[6], A. STEELMAN[6,7,3], C. BEST-POPESCU[1,4], G. POPESCU[1,4,5], M. CAPUTO[2], L. RUND[1,8], R. JOHNSON[1,6,8], T. DAS[9], M. KUCHAN[10]; [2]Div. of Nutritional Sci., [1]Univ. of Illinois Urbana-Champaign, Urbana-Champaign, IL; [3]Neurosci. Program, [4]Dept. of Bioengineering, [5]Dept. of Electrical and Computer Engin., Beckman Inst. for Advanced Sci. and Technol., Urbana, IL; [6]Div. of Nutritional Sci., Urbana, IL; [7]Dept. of Animal Sci., Urbana, IL; [8]Dept. of Animal Sci., Lab. of Integrative Immunol. & Behavior, Urbana, IL; [9]Discovery Res., [10]Strategic Res., Abbott Nutr., Columbus, OH

Abstract: Deficient myelination in the central nervous system is associated with neurodevelopmental complications, Alzheimer's disease and temporal lobe epilepsy. Furthermore, deficient remyelination is thought to underlie neurodegeneration in multiple sclerosis patients. Although both neurons and oligodendrocytes have been extensively studied individually, their interactions are still incompletely understood. New techniques are needed to further study the intricacies of this interplay in terms of cellular and molecular dynamics. Spatial Light Interference Microscopy (SLIM) is a quantitative imaging technique that generates phase maps related to the dry mass content of the sample. Recently, we have assessed myelin content in piglet brain tissue using color SLIM, which combines phase maps with corresponding color images. In this study, we examined the ability of SLIM to quantifying myelination at the single axon level. We imaged 18 sets of cocultures comprising hippocampal neurons and oligodendrocytes, of varying densities over two weeks. After imaging, the cocultures were fixed and stained for both myelin and intermediate filament, using antibodies specific to proteolipid protein (PLP) and neurofilament (NF) respectively. Registering the resulting immunofluorescent images with dry mass videos allowed the evaluation of myelin development throughout the course of neuron-oligodendrocyte intercourse. Preliminary analysis has shown that the proximity and contact of oligodendrocytes contributes to normal axonal mass and diameter. In summary, we have quantified myelin development from its inception in oligodendrocytes to its periodic insulation of axons. These results will provide insight on the details of myelin transport, as well as improve the sensitivity and validity of further myelin quantifications in neuronal cultures and brain tissues.

Disclosures: Y. Lee: None. **M. Fanous:** None. **A. Louie:** None. **A. Steelman:** None. **C. Best-Popescu:** None. **G. Popescu:** None. **M. Caputo:** None. **L. Rund:** None. **R. Johnson:** None. **T. Das:** None. **M. Kuchan:** None.

Poster

206. Molecular and Cellular Mechanisms of Demyelinating Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

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Topic: B.12. Demyelinating Disorders

Support: NIH Grant 1R01NS107523-01
National Multiple Sclerosis Society Grant (G-150805906)
CDMRP/DOD (W81XWH-17-1-0268)

Title: Tracking the evolution of CNS remyelinating lesion with neutral red dye

Authors: *M. BAYDYUK, D. S. CHA, J. HU, R. YAMAZAKI, E. M. MILLER, V. N. SMITH, J. K. HUANG;
Biol., Georgetown Univ., Washington, DC

Abstract: Animal models of central nervous system (CNS) demyelination, including toxin-induced focal demyelination and immune-mediated demyelination through experimental autoimmune encephalomyelitis (EAE), have provided valuable insights into the mechanisms of neuroinflammation and CNS remyelination. However, the ability to track changes in transcripts, proteins, and metabolites, as well as cellular populations during the evolution of a focal lesion, has remained challenging. Here, we developed a novel method to label CNS demyelinating lesions by the intraperitoneal injection of a vital dye, neutral red (NR) into mice before sacrifice. We demonstrate that NR labeled lesions can be easily identified on the intact spinal cord in both lysolecithin- and EAE-mediated demyelination models. Using fluorescence microscopy, we detected NR in activated macrophages/microglia and astrocytes, but not in oligodendrocytes present in lesions. Importantly, we successfully performed RT-qPCR, western blot, flow cytometry, and mass spectrometry analysis of precisely dissected NR labeled lesions at 5, 10, and 20 days post lesion (dpl) and found differential changes in transcripts, proteins, immune cell populations, and metabolites in lesions over the course of remyelination. Therefore, NR administration is a simple and powerful method to track and analyze the detailed molecular, cellular, and metabolic changes that occur within the lesion microenvironment over time following CNS injury. Furthermore, this method can be used to identify molecular and metabolic pathways that promote remyelination and facilitate the development of therapies to enhance this process in demyelinating disorders such as multiple sclerosis.

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Poster

206. Molecular and Cellular Mechanisms of Demyelinating Disorders

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Title: Gcn2 plays a protective role in a pharmacological demyelination model

Authors: *A. E. BRITO, P. FALCON, M. ESCANDON, C. JEREZ, Y. MARAMBIO, S. MATUS-MONTERO;
Fundación Ciencia Y Vida, Santiago de Chile, Chile

Abstract: The kinase general control nonderepressible 2 (GCN2), a nutrient stress sensor of the Integrated Stress Response signaling pathway, activates a variety of adaptive mechanisms to nutrient deficient diet through the phosphorylation of the eukaryotic translation initiation factor 2 alpha (eIF2α). One cell type that undergoes metabolic stress during it ontogeny is the oligodendrocyte glial cell, which have to express a great amount of myelin proteins to fulfill the correct axonal myelinaton in addition to providing energy substrates to neurons. Alterations in oligodendrocyte cells may end in neurodegenerative diseases with demyelinating features. If nutritional stress can modulate oligodendrocyte biology and function remains unknown. To answer this question, GCN2 deficient mice were challenged to remyelinate after acute demyelination induced by pharmacological intoxication. GCN2 deficient and control littermates WT mice were fed with regular chow with 0.2% of cuprizone (CPZ), a drug that specifically kills oligodendrocytes, during 5 weeks (5W) to achieve a robust demyelination, and then 1 week fed with normal chow to allow the remyelination progress (6W). Myelin recovery was assessed through protein levels examination of a conspicuous myelin protein MBP (Myelin Basic Protein), through western blotting and immunohistochemistry. GCN2 deficient mice are not able to fulfill myelination after one week of normal chow feeding as WT mice do. MBP levels in GCN2 deficient mice after remyelination period (6W) remains comparable with the MBP levels observed in the demyelinated mice after 5W CPZ treatment. GCN2 deficient mice fail to remyelinate after the pharmacological intoxication, suggesting that GCN2 is involved in oligodendrocyte physiological function during remyelination, hence playing a protective role during acute remyelination of the CNS.

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Poster

206. Molecular and Cellular Mechanisms of Demyelinating Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 206.17/C64

Topic: B.12. Demyelinating Disorders

Title: Small molecule inhibition of the muscarinic M1 acetylcholine receptor by potent, selective antagonists facilitates OPC differentiation

Authors: *M. M. POON[1], K. I. LORRAIN[1], A. LORENZANA[1], K. J. STEBBINS[1], A. BROADHEAD[1], T. SCHRADER[2], Y. XIONG[2], J. BACCEI[2], A. J. GREEN[4], J. R. CHAN[4], D. S. LORRAIN[3];
[1]Biol., [2]Chem., [3]Pipeline Therapeut., San Diego, CA; [4]Neurol., UCSF, San Francisco, CA

Abstract: Inhibition of the muscarinic acetylcholinergic receptors by non-selective muscarinic antagonists (e.g., clemastine, benztropine) accelerates the differentiation of oligodendrocyte precursor cells (OPCs) into oligodendrocytes (OLs). Subsequent work has implicated the M1 isoform as being a key driver of this phenomenon. In-house chemistry efforts have identified a number of potent, selective M1 antagonists. Using these, we have characterized the effects of inhibiting M1 in a diverse set of *in vitro* assays, including OPC differentiation, cortical myelination, and organotypic brain slice. Our data show that a selective, small molecule inhibitor of M1 is sufficient to drive OPCs towards differentiation into oligodendrocytes that express markers like myelin basic protein. Moreover, these OLs are functional, i.e., capable of axonal wrapping and induction of nodes of Ranvier. Of note, an M3 selective antagonist (Sagara et al., 2006) was not active in a rat OL differentiation assay. In concert with our *in vivo* data (also presented at this meeting), a strong case can be made that the development of an M1 selective small molecule antagonist is a promising approach for treating demyelinating diseases such as multiple sclerosis.

Disclosures: **M.M. Poon:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. **K.I. Lorrain:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. **A. Lorenzana:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. **K.J. Stebbins:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. **A. Broadhead:** A.

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Poster

206. Molecular and Cellular Mechanisms of Demyelinating Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 206.18/C65

Topic: B.12. Demyelinating Disorders

Title: PIPE-359, a novel, potent and selective muscarinic M1 receptor antagonist as a therapeutic approach for remyelination in multiple sclerosis

Authors: *K. J. S. STEBBINS[1], M. M. POON[1], A. BROADHEAD[1], G. C. EDU[1], A. LORENZANA[1], K. I. LORRAIN[1], T. SCHRADER[1], Y. XIONG[1], J. BACCEI[1], C. BACCEI[1], A. GREEN[2], J. R. CHAN[3], D. S. LORRAIN[1];
[1]Pipeline Therapeutics, Inc., San Diego, CA; [3]Neurol., [2]UCSF, San Francisco, CA

Abstract: Novel small molecule approaches aimed at stimulating remyelination would greatly complement immunotherapies and provide significant neural protection in demyelinating conditions such as multiple sclerosis (MS). Recently, we described the muscarinic M1 receptor (M1R) as an important regulator of oligodendrocyte precursor cell (OPC) differentiation and a promising target for drug discovery. We developed PIPE-359, a novel, potent and selective M1R antagonist and highlight it’s potential for remyelination. PIPE-359 binds with high affinity to the M1 receptor and demonstrates selectivity relative to the other muscarinic receptors (M2, M3, M4 and M5). PIPE-359 promotes differentiation of rodent OPCs and increases myelination in

cultured rat brain slices. This compound has good oral exposure and brain penetration. PIPE-359 demonstrates good *in vivo* M1R occupancy and functional inhibition of M1R signaling as determined by IP1 accumulation. At dose levels which occupy the M1R, we demonstrate remyelination potential in both cuprizone and myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalitis (EAE) models of demyelination. In the EAE model this is accompanied by improvement in clinical behavior score. Furthermore, PIPE-359 exhibits marked improvement of EAE-induced changes in visual evoked potential N1 latency (presented in more detail at this meeting in the poster by Edu *et al*). These data highlight the therapeutic potential of a selective M1R antagonist to benefit conditions such as MS in which demyelination plays a role.

Disclosures: K.J.S. Stebbins: A. Employment/Salary (full or part-time):; Pipeline Therapeutics, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics, Inc. **M.M. Poon:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics, Inc. **A. Broadhead:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics, Inc. **G.C. Edu:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics, Inc. **A. Lorenzana:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics, Inc. **K.I. Lorrain:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics, Inc. **T. Schrader:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics, Inc. **Y. Xiong:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics, Inc. **J. Baccei:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics, Inc. **C. Baccei:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics, Inc. **A. Green:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics, Inc. **J.R. Chan:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics, Inc. **D.S. Lorrain:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of

intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics, Inc..

Poster

206. Molecular and Cellular Mechanisms of Demyelinating Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 206.19/C66

Topic: B.12. Demyelinating Disorders

Title: The muscarinic M1 antagonist PIPE-359 demonstrates remyelination *in vivo* through visual evoked potential (VEP) and electron microscopy (EM) in mice with experimental autoimmune encephalitis (EAE)

Authors: *G. C. EDU[1], K. J. STEBBINS[1], A. R. BROADHEAD[1], M. M. POON[1], A. O. LORENZANA[1], T. O. SCHRADER[1], Y. XIONG[1], J. BACCEI[1], A. J. GREEN[2], J. R. CHAN[2], D. S. LORRAIN[1];
[1]Pipeline Therapeut., San Diego, CA; [2]Dept. of Neurol., Univ. of California San Francisco, San Francisco, CA

Abstract: Multiple sclerosis is characterized by immune mediated myelin injury and progressive axonal loss. In patients, remyelination is limited and is inadequate to fully restore neuronal function. Targeting a molecular mechanism which regulates oligodendrocyte differentiation and remyelination is a promising therapeutic approach. The muscarinic M1 receptor has been identified as a target for remyelination through differentiation of oligodendrocyte precursor cells (OPCs) into oligodendrocytes. PIPE-359 is a novel, potent and selective M1 antagonist with good oral exposure and brain penetration which is efficacious in rodent models of demyelination such as cuprizone and experimental autoimmune encephalitis (EAE). Visual evoked potential (VEP) is a clinically translatable model used in patients with multiple sclerosis due to its ability to measure myelin damage of the visual pathway as determined by the latency in the VEP waveform. Flash VEPs were recorded prior to EAE induction and then again at 7, 14, and 21 days post-induction. Animals were treated with vehicle or PIPE-359 starting on day 0. Upon study termination, spinal cords and optic nerves were collected and processed for EM, and the resulting images were analyzed to determine g-ratios. In vehicle treated animals, VEP N1 latency increased significantly over time relative to non-EAE controls whereas PIPE-359 treated mice showed little to no change in VEP N1 latency out to 21 days. Analysis of the spinal cord and optic nerve EM images and the calculated g-ratios confirm that PIPE-359 increases remyelinated axons *in vivo* and this correlates with the VEP results and the behavioral clinical score. These preclinical studies provide further support for developing a selective M1 antagonist for the treatment of demyelinating diseases such as multiple sclerosis.

Disclosures: **G.C. Edu:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. **K.J. Stebbins:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. **A.R. Broadhead:** A. Employment/Salary (full or part-time):; PIpeline Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. **M.M. Poon:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. **A.O. Lorenzana:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. **T.O. Schrader:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. **Y. Xiong:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. **J. Baccei:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. **A.J. Green:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. F. Consulting Fees (e.g., advisory boards); Pipeline Therapeutics. **J.R. Chan:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics. F. Consulting Fees (e.g., advisory boards); Pipeline Therapeutics. **D.S. Lorrain:** A. Employment/Salary (full or part-time):; Pipeline Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pipeline Therapeutics.

Poster

206. Molecular and Cellular Mechanisms of Demyelinating Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 206.20/C67

Topic: B.12. Demyelinating Disorders

Support: TLOAF Grant 2018

Title: Cell modeling and assay development for Krabbe disease

Authors: A. MANAVALAN, A. HERDT, W. CHING, *C. LEE;
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Abstract: Loss-of-function mutations in galactocerebrosidase (GALC) lead to the development of Krabbe disease, a degenerative disorder characterized by demyelination and neuroinflammation. Several pathogenic missense mutations have been identified in the *GALC* gene of Krabbe patients; which impair GALC function by altering the catalytic site and/or protein trafficking. In these patients, GALC protein may be present but inactive or missing from the lysosome. However, most mutant proteins will retain some catalytic activity if they can be stabilized, properly folded and/or transported to the lysosome. Pharmacological chaperones (PC) are an emerging class of small-molecule therapeutics that can stabilize protein structure and restore enzyme function. We previously identified a PC candidate for Krabbe disease, α-lobeline (LB), which has been shown to increase the activity of several GALC mutants (D544N, G553R, E130K, N295T and G57S). We hypothesize that *GALC* missense mutations are amenable to functional correction by LB and other PC candidates.
To characterize the effect of disease-related mutations on GALC function, we performed expression studies in HEK293 cells and MO3.13 cells, a human oligodendrocyte cell line, to analyze intracellular and extracellular GALC levels and activity. Furthermore, to examine the PC-effects of LB, we measured the levels of lysosomal and secreted GALC mutants after treatment. Compared to WT-GALC, the disease-related missense mutations we studied induced GALC mistrafficking as measured by reduced secretion, accumulation of precursor protein and/or reduction of lysosomal GALC, which led to significant reduction of enzymatic activity. LB treatment on D544N-GALC expression increased the secretion and lysosomal levels of GALC compared to vehicle treated cells. Overall, we have undertaken a comprehensive characterization of GALC molecular phenotypes, including lysosomal localization, secretion and enzymatic activity, in order to establish the pathogenicity of GALC mutations and identify PC targets for future *in vivo* efficacy studies.

Disclosures: A. Manavalan: None. **A. Herdt:** None. **W. Ching:** None. **C. Lee:** None.

Poster

206. Molecular and Cellular Mechanisms of Demyelinating Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 206.21/C68

Topic: B.12. Demyelinating Disorders

Title: Developing better biomarkers to track demyelination *in vivo* in a cuprizone mouse model

Authors: E. DEFENSOR, M. LIM, ***L. SCHAEVITZ**;
Vium, San Mateo, CA

Abstract: The cuprizone mouse model is frequently used to study non-T cell dependent mechanisms of de/remyelination and the promyelinating activity of discovery compounds. Demyelination is associated with deficits in motor function which are often tracked over time using various standard behavioral tests. The findings reported from these tests are highly variable. For example, studies evaluating 0.2% cuprizone treated animals in the open field report activity that is either increased, decreased, or normal compared to controls.
In the current study, we hypothesize that continuous collection of activity data in the home cage throughout the study will allow detection of subtle changes in motor function resulting from demyelination. Female C57Bl/6J mice were single housed on racks fitted with video cameras connected to the cloud. After one week of acclimation, animals were randomly assigned to one of two groups that received either control chow (n = 6) or chow with 0.2% cuprizone (n = 6) for 6 weeks. In the cloud, computer algorithms processed the video to create a motion biomarker at 24 frames/s.
Analysis of motion revealed variable responses depending on the time of day (day vs night) and recent procedures (cage change). Nighttime motion was significantly decreased in cuprizone treated compared with control mice on nights 5 - 14, 20 - 28, and 35 - 41. Daytime motion was not significantly different between cuprizone and control mice with the exception of the day of cage changes. Daytime motion was significantly elevated in cuprizone-treated mice vs. controls on cage change day 14.
As expected, continuous automated collection of motion during the nighttime, when mice are generally most active, allowed for high-throughput detection of changes in motor function, which may be associated with demyelination. In addition, motion data corroborated the various results reported in the literature such as hyperactivity in novel environments (i.e. cage change) and normal activity during the daytime.
The current results reinforce our understanding that traditional behavioral tests, utilizing a limited window of observation, are more influenceable by factors such as novelty and environmental disturbances. In contrast, continuous data collection allows for a more accurate characterization of an animal model since transient disturbances are detected as anomalies within the larger data set. Taken together, the results highlight the utility of the continuous automated collection of undisturbed behavior in detecting even subtle changes associated with demyelination in the cuprizone mouse model and further suggests the increased utility of these methods for additional models.

Disclosures: **L. Schaevitz:** A. Employment/Salary (full or part-time):; Vium. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Vium. **E. Defensor:** A. Employment/Salary (full or part-time):; Vium. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Vium. **M. Lim:** A. Employment/Salary (full or part-time):; Vium. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Vium.

Poster

206. Molecular and Cellular Mechanisms of Demyelinating Disorders

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 206.22/C69

Topic: B.12. Demyelinating Disorders

Title: Oligodendrogenesis from neural stem cells is inhibited by GPNMB

Authors: *D. Z. RADECKI[1], J. SAMANTA[2];
[1]Sch. of Vet. Med., Univ. of Wisconsin-Madison, Madison, WI; [2]Sch. of Vet. Med., Univ. of Wisconsin Madison, Madison, WI

Abstract: Regenerating oligodendrocytes and myelin in the central nervous system (CNS) is critical to ameliorate demyelinating diseases such as multiple sclerosis (MS). Neural stem cell (NSC) residing in the ventral subventricular zone (vSVZ) is a source of new oligodendrocytes. These NSCs express the transcription factor Gli1 and have been shown to migrate to areas of demyelination and differentiate into oligodendrocytes. Inhibition of Gli1 enhances their migration and differentiation upon demyelination, while in healthy brains the Gli1 NSCs continue to produce neuroblasts that migrate to the olfactory bulb. We performed a transcriptome analysis of the Gli1 NSCs under control and demyelinating conditions and identified a potential regulator of NSC derived oligodendrogenesis, glycoprotein non-metastatic melanoma b (GPNMB). GPNMB is a single pass transmembrane protein expressed in NSCs of the developing and mature mouse CNS, and is not expressed in oligodendrocyte or oligodendrocyte precursor (OPC) cells in healthy brains. In NSCs lacking Gli1, GPNMB expression is decreased on demyelination resulting in increased migration and oligodendrogenesis. This shows GPNMB is a negative regulator of remyelination, which we confirmed *in vitro* through overexpression of GPNMB in NSCs which resulted in a decrease in the number of oligodendrocyte lineage cells. Further, TGFβ-1, could increase expression of GPNMB while other known modulators of NSCs such as sonic hedgehog (Shh) had no effect. Together, these results show that GPNMB is regulated by TGFβ-1 to inhibit NSC derived oligodendrogenesis.

Disclosures: D.Z. Radecki: None. **J. Samanta:** None.

Poster

207. Demyelinating Disorders: Human and Animal Studies and Therapeutics

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 207.01/C70

Topic: B.12. Demyelinating Disorders

Support: Indiana University of Pennsylvania (Biology Department and School of Graduate Studies and Research)
Pennsylvania Academy of Science
Saint Vincent College

Title: Effects of a modified ketogenic diet on motor and cognitive function, peptidylarginine deiminase 2 expression, neural and immune cells in the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis

Authors: J. WILLIS[1,2], B. BETHKE[2], *D. V. WIDZOWSKI[1];
[1]Biol., Indiana Univ. of Pennsylvania, Indiana, PA; [2]Biol., St. Vincent Col., Latrobe, PA

Abstract: Multiple sclerosis (MS) is a demyelinating, inflammatory, neurological autoimmune disease of the Central Nervous System, with no established cause or cure. Patients experience symptoms ranging from optic neuritis to weakness, vertigo, numbness, tremor, paralysis and cognitive dysfunction. Current pharmaceuticals are effective for some MS patients but not all, frequently require injections/infusions and many cause significant side effects. Dietary interventions are noninvasive and may have as profound an impact on MS pathophysiology as prescriptions but without the negative side effects. In an exploratory study, we tested this hypothesis using the Experimental Autoimmune Encephalomyelitis (EAE) model of MS in which ten randomized female, 9-week-old C57BL/6 mice were fed either a rodent standard diet (SD) (n=5) or a modified ketogenic diet (mKD) (n=5) rich in medium-chain triglycerides and omega-3-6-9 fatty acids. Both groups consumed their assigned dietary regimes for the duration of the 8-week study, and baseline observations and metrics were recorded during the 3-week prophylactic period prior to EAE immunization. Behavioral assessments were performed before and during the experimental portions and gene expression and epigenetic analyses were performed at the end of the study. Compared to SD controls that exhibited substantial clinical signs, the mKD delayed EAE onset and significantly decreased disease severity (reduced clinical scores). EAE-induced cognitive deficits were also reduced in the mKD cohort. Mice fed the mKD showed downregulated *Peptidylarginine deiminase 2 (Padi2)* expression in brain and spinal cord relative to controls. This study demonstrated the beneficial potential of the mKD in the EAE model. Ongoing follow-up experiments will confirm (with a larger sample size, n=10 per group) and expand the analysis of the effects of the mKD on the EAE model. Specifically, characterizing the effects of the mKD on neuroimmune mechanisms involved in autoimmunity within the brain, spinal column, and optic nerves including analysis of markers for immune cells (e.g., leukocytes and macrophages) and neural cells (e.g., myelin, oligodendrocytes, damaged axons, and surviving neurons). Additionally, we are evaluating the effect of sucrose added to the mKD to assess the effect of a higher carbohydrate content on immune and neural responses in the EAE model. These studies will further test the hypothesis that ketogenic diets may promote beneficial outcomes such as neuroprotection and reduced inflammation in the EAE model of MS.

Disclosures: D.V. Widzowski: None. **J. Willis:** None. **B. Bethke:** None.

Poster

207. Demyelinating Disorders: Human and Animal Studies and Therapeutics

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 207.02/C71

Topic: B.12. Demyelinating Disorders

Support: Hilldale Undergraduate Research Fellowship

Title: Gli2 is necessary for remyelination by ventral neural stem cells

Authors: *H. MESSLING[1], D. Z. RADECKI[2], J. HAGGERTY-SKEANS[3], J. L. SALZER[3], J. SAMANTA[4];
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Abstract: Enhancing repair of myelin is an important therapeutic goal in many neurological disorders characterized by demyelination. In the healthy adult brain, the ventral neural stem cells (vNSCs) in the subventricular zone (SVZ), marked by expression of Gli1 migrate to the olfactory bulb to produce interneurons and astrocytes but do not generate oligodendrocytes in the brain. However, in the presence of demyelination, they migrate to the white matter lesions and generate oligodendrocytes; inhibition of Gli1 further increases their contribution to remyelination. Gli1 and Gli2 are both transcriptional effectors of the sonic hedgehog pathway with highly conserved domains but the role of Gli2 in remyelination by vNSCs is not clear. Here we define the function of Gli2 in remyelination by endogenous vNSCs in the adult mouse SVZ. We found that inhibition of Gli1 leads to upregulation of Gli2 in the vNSCs upon demyelination suggesting a positive role in remyelination. Further, genetic ablation of Gli2 specifically in the $Gli1^{NULL}$ vNSCs results in inhibition of migration of these cells to the demyelinating lesion with a concomitant increase in migration to the olfactory bulb and also reduces their differentiation into mature oligodendrocytes in the lesions. Thus, Gli2 plays an essential role in differentiation and migration of vNSCs in the lesions for remyelination.

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Poster

207. Demyelinating Disorders: Human and Animal Studies and Therapeutics

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 207.03/C72

Topic: B.12. Demyelinating Disorders

Support: ELA GRANT 2015-010C1A

Title: Brain targeted and biodegradable nanoparticles for enzyme replacement therapy in a Krabbe disease mouse model

Authors: *A. DEL GROSSO[1], M. GALLIANI[2], L. ANGELLA[1], M. SANTI[3], I. TONAZZINI[1], G. PARLANTI[1], G. SIGNORE[4], M. CECCHINI[1];
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Abstract: Lysosomal storage disorders (LSDs) are a large group of metabolic diseases, individually rare but collectively common (1:5,000 live births). Usually, they result from an enzyme deficiency within lysosomes, which ultimately causes accumulation of undegraded substrates. The most clinically applied method to treat LSDs is the systemic administration of the missing enzyme. This approach, however, is not effective in the case of LSDs that involve the central nervous system (CNS); the presence of the blood brain barrier (BBB), in fact, forbids translocation of big molecules into the brain. Here, a new enzyme delivery system based on the encapsulation of cross-linked enzyme aggregates (CLEAs) into poly-(lactide-co-glycolide) (PLGA) nanoparticles (NPs) functionalized with brain targeting peptides (Ang2, g7 or Tf2) is demonstrated for Krabbe disease (KD or Globoid cell leukodystrophy; OMIM #245200), an inherited neurodegenerative LSD caused by the genetic deficiency of the enzyme galactosylceramidase (GALC; EC 3.2.1.46). We firstly synthesize and characterize Ang2, g7 and Tf2-targeted GALC CLEA NPs. Then, we study NP cell uptake and trafficking, assessing their capability to reinstate enzymatic activity *in vitro*. Finally, we successfully test our NP formulations in the Twitcher mouse, the spontaneous murine model of KD. We report enzymatic activity measurements in the nervous system and in typical accumulation districts upon NP intraperitoneal injections, demonstrating GALC activity recovery in the brain up to the level of unaffected control mice. In addition, the presence of targeted NPs in the brain was confirmed by confocal microscopy. Taken together, these results open new therapeutic perspectives for KD, and for all LSDs with major involvement of the CNS.

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Poster

207. Demyelinating Disorders: Human and Animal Studies and Therapeutics

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 207.04/C73

Topic: B.12. Demyelinating Disorders

Title: What is "conventional therapy"? A 10-years systematic review on physical rehabilitation in multiple sclerosis

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Abstract: Background. In controlled studies on physical rehabilitation, the term “conventional therapy” is generally used in referring to the non-experimental group. It seems to indicate a unique and usual practice, commonly known and widely accepted. However, what is “conventional therapy” in practice? Our aims were to determine whether exists a univocal and homogeneous definition of this expression, to analyze its meaning and to define its specific features. To do so, we focused on the physical rehabilitation for people with multiple sclerosis (MS) and we systematically reviewed published clinical trials on this topic. **Methods.** A systematic literature search was conducted using MEDLINE from 2008 to 2018. Inclusion criteria were: controlled clinical trials on MS physical rehabilitation; “conventional therapy” or synonyms that are commonly used to classify the treatment program in the control group (e.g., “traditional physical therapy”, “standard rehabilitation program”, “conventional rehabilitation”); people with MS recruited both in experimental group and in control group. **Results.** Of 884 references identified through electronic searching, 122 were eligible for evaluation. The 84% of papers did not fulfil the inclusion criteria. One third of these did not provide a specific description for “conventional therapy” or synonyms. Thus, we totally screened 19 clinical trials. Only 2 studies specified the use of the Consolidated Standards of Reporting Trials (CONSORT) Statement guidelines. The present review enabled to highlight a situation of heterogeneity in the definition of “conventional therapy”, including a multitude of interventional strategies, with differences in volumes and intensities of therapies. A remarkable difference was also found in the number of interventions assigned as conventional rehabilitation, which ranged from 1 to 7; the 58% of studies administered at least 4 treatments. This heterogeneity was reflected in studies that took place in different countries, as well as in studies conducted within the same country, emphasizing the lack of a common line of intervention also at national level. **Conclusion.** To

date, the umbrella term “conventional therapy” seems not to provide a univocal definition for the control treatment in MS physical rehabilitation trials. This heterogeneity in definition and specific features may lead to reduce the quality of the studies, limiting the comparability between trials and decreasing the reproducibility of the protocol. The present review focuses on the need to find new standardised, uniform and shared protocols for MS conventional rehabilitation.

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Poster

207. Demyelinating Disorders: Human and Animal Studies and Therapeutics

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 207.05/C74

Topic: B.12. Demyelinating Disorders

Support: Department of Defense Grant: W81XWH-16-1-0351

Title: Progressive multiple sclerosis demonstrates greater sensorimotor function disparity between upper and lower extremities compared to relapsing-remitting and controls

Authors: *S. SATO[1], J. BUONACCORSI[2], J. D. MIEHM[3], C. RAJALA[3], J. LIM[4], J. A. KENT[3], R. E. A. VAN EMMERIK[3];
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Abstract: INTRODUCTION: Multiple sclerosis (MS) is a demyelinating disease of the central nervous system that can be categorized into relapsing-remitting (RRMS) and progressive subtypes (PMS). Prior work suggests that lower extremity motor function often is more impaired than that of the upper extremities in MS, but it is not yet known whether this difference extends to sensory function, and is consistent between MS subtypes.
OBJECTIVES: To quantify upper- and lower extremity sensorimotor function and determine whether differences between the extremities vary among RRMS and PMS subtypes and non-MS controls (CON).
METHODS: 32 RRMS (3 males, 54±10 y; mean±SD), 31 PMS (11 males, 60±8 y) and 30 CON (6 males, 55±12 y) were evaluated for vibration perception threshold (VPT; Volts) and motor function on all hands and feet. VPT values were averaged for three hand (thumb, index, base of palm) and three foot (big toe, head of 5th metatarsal, and heel) locations. Motor function was assessed as the number of rapid hand or foot taps in 10 s. The main outcome measures were the difference scores (Δ) for upper and lower extremity sensory (hand VPT – foot VPT) and motor (number of hand – foot taps) function.

RESULTS: Hand VPT was lower and hand-tap count was higher in CON compared to both RRMS (VPT: $p = 0.005$; Taps: $p < 0.001$) and PMS (VPT: $p = 0.002$; Taps: $p < 0.001$), with no differences between the MS subtypes (VPT: $p = 0.094$; Taps: $p = 0.403$). Foot VPT was lower and foot-tap count was higher in CON compared to RRMS (VPT: $p = 0.035$; Taps: $p < 0.001$) and PMS (VPT: $p < 0.001$; Taps: $p < 0.001$), as well as RRMS compared to PMS (VPT: $p < 0.001$; Taps: $p = 0.002$). For all groups, VPT in the hand was lower than in the foot (CON: -6.5 ± 1.9, $\Delta \pm 2SE$; RRMS: -9.6 ± 3.5; PMS: -20.3 ± 5.0; all ps < 0.001), and tap count was higher in the hand than in the foot (CON: 16.9 ± 2.1; RRMS: 15.7 ± 3.3; PMS: 22.1 ± 3.4; all ps < 0.001). There were group x extremity interactions for ΔVPT ($p < 0.001$) and Δtaps ($p = 0.018$): PMS had a larger ΔVPT than RRMS ($p = 0.001$, 95% CI = [-16.8, -4.6]) and CON ($p < 0.001$, CI = [-19.2, -8.3]), whereas ΔVPT for RRMS did not differ from CON ($p = 0.126$, CI = [-7.1, 0.9]). Likewise, Δtap count was larger in PMS than RRMS ($p = 0.010$, CI=[1.6, 11.1]) and CON ($p = 0.012$, CI = [1.2 9.2]), while RRMS did not differ from CON ($p = 0.564$, CI = [-5.0, 2.8]). CONCLUSION: Hand and foot sensorimotor function differed between MS groups and CON. Only foot function differed between MS subtypes. For all groups, hand sensory and motor function were better than foot function. Notably, these extremity differences were larger in PMS than in RRMS and CON, suggesting that greater demyelination in the longer axon tracts supplying the lower extremities may sustain damage in PMS.

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Poster

207. Demyelinating Disorders: Human and Animal Studies and Therapeutics

Location: Hall A

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Program #/Poster #: 207.06/C75

Topic: B.12. Demyelinating Disorders

Support: CNPQ
FAPERJ
PROEX

Title: Myelin breakdown favors mycobacterium leprae survival in Schwann cells

Authors: *B. J. DE SOUZA[1], E. N. SARNO[1], F. A. LARA[1], B. S. MIETTO[2];
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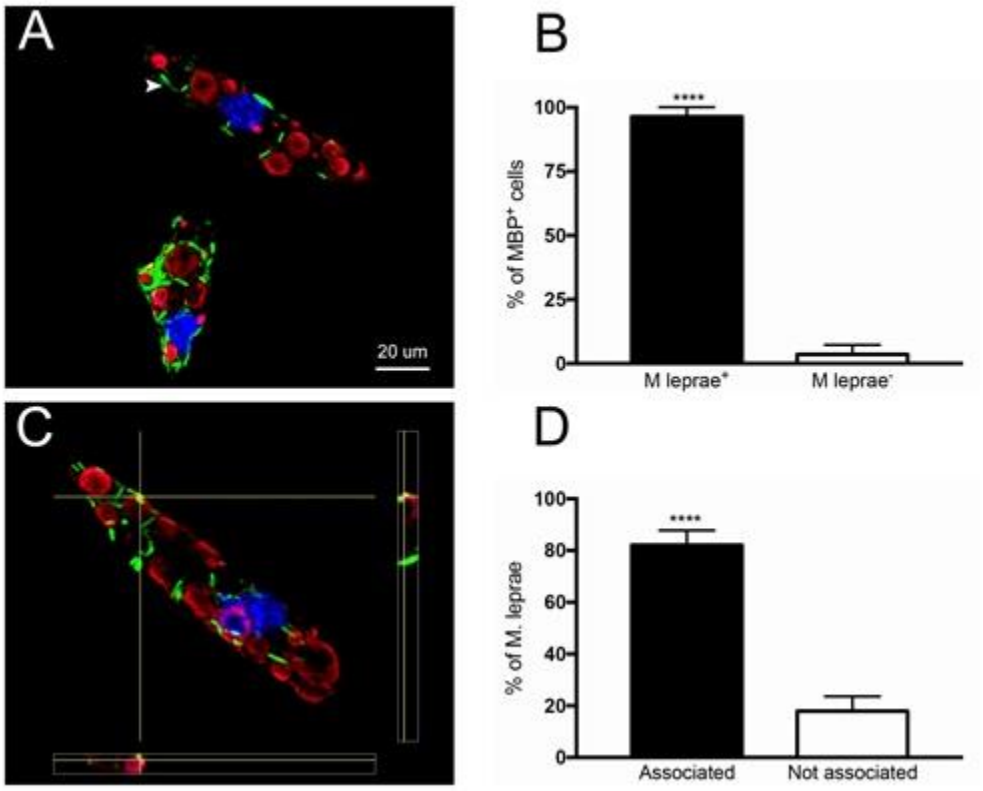
Abstract: Leprosy neuropathy is a chronic degenerative infectious disorder of the peripheral nerve caused by the intracellular obligate pathogen Mycobacterium leprae (*M. leprae*). Among all non-neuronal cells that constitute the nerve, Schwann cells are remarkable in supporting *M.*

leprae persistence intracellularly. Notably, the success of *M. leprae* infection in myelinated Schwann cells has been attributed to its ability in triggering the demyelination phenotype. However, the exactly role *M. leprae* plays during the ongoing process of myelin breakdown inside Schwann cells is entirely unknown. Here, we provided evidence showing close association of *M. leprae* with degenerating myelin profiles in Schwann cells *in vitro* and *in vivo*. In addition, *M. leprae* infection accelerated the rate of myelin breakdown and clearance in Schwann cells, by modulating a set of regulatory genes involved in myelin maintenance and autophagy. Additionally, we also found increased levels of lipid droplets in infected Schwann cells if compared to uninfected ones. Interestingly, when we prevented myelin from degradation, we dramatically reduced *M. leprae* viability inside Schwann cells, corroborating the paramount role of myelin breakdown-derived elements favoring *M. leprae* survival inside Schwann cells. Collectively, our study provided novel evidence of the critical role of myelin breakdown as a lipid source favoring *M. leprae* viability in Schwann cells, and may explain the demyelination phenotype as an evolutionary conserved mechanism trigged by *M. leprae* to survive chronically in the peripheral nerve.



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Poster

207. Demyelinating Disorders: Human and Animal Studies and Therapeutics

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Program #/Poster #: 207.07/C76

Topic: B.12. Demyelinating Disorders

Support: HMRF-05163156

Title: Derivation of human bone marrow -derived glial progenitor cells for remyelination therapy

Authors: Y.-P. TSUI[1], G. LAM[1], K. WU[1], Y.-S. CHAN[1], D. K.-Y. SHUM[1], ***G. K.-H. SHEA**[2]; [1]Sch. of Biomedic. Sci., Fac. Med., Univ. Hong Kong, Hong Kong, China; [2]Dept. Orthopaedics & Traumatology., Fac. Med., Univ. Hong Kong, Hong Kong, China

Abstract: CNS myelination is facilitated by oligodendrocytes (OL). Disorders of myelin formation can be congenital or acquired and result in significant functional impairment. Congenital disorders of myelin formation are often fatal and pose as an attractive disease model for cell replacement therapy. We have previously been able to generate glia from rat and human bone marrow, as a means towards autologous cell therapy. In this study, we utilised our glial induction protocol to derive glial progenitor cells (GPCs) from bone marrow stromal cell (hBMSC) harvested from human diaphyseal marrow specimens. The generated hBMSC-GPCs were highly enriched in OPC markers expression, including OLIG-2, PDGFRα, NG2, SOX-10 and O4. Following transplantation into the myelin-deficient shiverer mouse brain, engrafted hBMSC-GPCs demonstrated the capacity for myelination as well as migration along white matter tracts. Both structural and functional rescue from hypomyelination was observed as hBMSC-OPC transplantation significantly extending mice lifespan, body weight, and motor function. No tumourogenicity was observed in mice sacrificed up to 5 months after transplantation. Our platform for generating myelinating glia from human bone marrow is rapid and efficient and promises a means of utilizing autologous cell therapy to treat demyelinating diseases and neurotrauma. Specifically, we circumvent hurdles to clinical translation associated with genetic reprogramming and narrow transplantation window periods.

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Poster

207. Demyelinating Disorders: Human and Animal Studies and Therapeutics

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 207.08/C77

Topic: B.12. Demyelinating Disorders

Support: Blusson Integrated Cure Partnership

Title: Effects of ketogenic diet on remyelination in a cuprizone/rapamycin model of demyelination

Authors: *N. ALAEIILKHCHI[1], K. KOLEHMAINEN[1], O. SEIRA[2], W. T. PLUNET[3], J. LIU[1], W. TETZLAFF[4];
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Abstract: Multiple sclerosis (MS) is characterized by demyelination and axonal injury/degeneration. Demyelinated axons are thought to be more susceptible to degeneration, due to exposure to the inflammatory environment and metabolic stress. Therefore, promoting remyelination is a promising therapy, as it might mitigate axonal loss. As opposed to pharmacological therapies, diet-based treatments have fewer side-effects. Ketogenic diet (KD) (high in fat, adequate in protein, while very low in carbohydrates) increases ketone body production and decreases inflammation, which is thought to facilitate remyelination. KD is currently being used clinically for some treatment resistant epilepsy, but its efficacy in MS models is unknown. Our objective is to assess the effects of KD on remyelination efficacy and oligodendrocyte differentiation in the cuprizone/rapamycin (cup/R) model of demyelination. We hypothesize that KD will promote oligodendrocyte differentiation and increase remyelination as compared to standard diet (SD). Demyelination was induced by feeding 10 weeks old C57BL6/J mice 0.3% cuprizone in their diet for the duration of 6 weeks accompanied by rapamycin injections. Experimental animals were divided into 2 groups: (1) standard diet (SD), (2) ketogenic diet (KD). The SD and KD groups received their respective diets from day 1 to 9 after cup/R cessation. Animals were sacrificed 10 days-post-cup/R and the cerebral hemispheres split for analysis: one half has been processed for immunohistochemistry and the other half is being subjected to electron microscopy analysis. Tissue samples are currently analyzed for myelin markers (MBP, PLP, CNPase) and oligodendrocyte lineage cell markers (Olig2, PDGFRalpha, NG2, and CC1), myelin pathology (i.e. remyelination, myelin debris, etc.) and general histopathological features (e.g. inflammation, microgliosis, etc.).

Disclosures: **N. Alaeiilkhchi:** None. **K. Kolehmainen:** None. **O. Seira:** None. **W.T. Plunet:** None. **J. Liu:** None. **W. Tetzlaff:** None.

Poster

207. Demyelinating Disorders: Human and Animal Studies and Therapeutics

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 207.09/C78

Topic: B.12. Demyelinating Disorders

Support: Dell Medical School Startup Funding

Title: Moderate alcohol consumption affects the gut microbiome in a murine multiple sclerosis model

Authors: *C. MAGUIRE[1], B. CASLIN[2,1], A. KARMAKAR[1], K. HELMSDOERFER[1], K. MOHLER[1], J. WARD[1], D. WYLIE[3], E. MELAMED[1];
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Abstract: Multiple Sclerosis (MS) is the leading cause of non-traumatic neurological disability in young adults. Environmental factors such as diet and the gut microbiome are known to be critical players in the pathogenesis of MS. Alcohol, as a dietary factor, is well known for its immunosuppressive effects and has been implicated in MS in epidemiological studies. However, the role of alcohol in influencing the gut microbiome and neuroinflammation in MS is not well understood. We hypothesized that moderate dose of alcohol could be protective in MS via changes to the gut microbiota. To evaluate this hypothesis, C57BL/6 male and female mice were fed either a 2.6% Lieber-deCarli alcohol diet or an isocaloric pair-fed diet for 4 weeks, followed by EAE induction with $MOG_{35-55\ and}$ pertussis and continued on respective diets for 44 days. Fecal samples were collected prior to alcohol diet initiation, prior to EAE induction, and 3 additional timepoints over the course of EAE. The microbial DNA was extracted and sequenced with 16S rRNA sequencing. Alpha and beta diversity indexes, hierarchical clustering, and bacterial relative abundances were calculated and visualized in Qiime and R. We applied a novel computational analysis to evaluate gut microbiome network interactions by grouping taxa that change similarly over time.Alcohol-fed males experienced significantly greater disease remission compared to alcohol-fed females and pair-fed controls. Beta diversity indexes revealed both a significant treatment group effect as well disease activity time-point effect. Thealcohol diet resulted in several sex-specific alterations in key microbiota known for their regulatory immune roles, including *Turicibacter*, *Akkermansia*, *Prevotella*, and *Clostridium*. Bacterial network analysis identified several unique bacterial modules that are significantly up-regulated in the alcohol-fed male mice compared to controls, comprised of Clostridiales taxa known to be protective in EAE and to modulate in response to Glatiramer Acetate.We present novel evidence that alcohol as a dietary factor can alter the course of neuro-inflammation in EAE in a sex

specific manner. Our data suggest that moderate alcohol consumption may be protective in males by impacting the composition of the gut microbiome.

Disclosures: C. Maguire: None. **B. Caslin:** None. **A. Karmakar:** None. **K. Helmsdoerfer:** None. **K. Mohler:** None. **J. Ward:** None. **D. Wylie:** None. **E. Melamed:** None.

Poster

207. Demyelinating Disorders: Human and Animal Studies and Therapeutics

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 207.10/C79

Topic: B.12. Demyelinating Disorders

Support: LLU-GRASP

Title: Exploring the physical and behavioral effects of a potassium channel antagonist in young adult male rats

Authors: *A. D. TROFIMOVA[1], P. S. GIFFORD[2], D. A. HESSINGER[2], D. L. BELLINGER[2], R. E. HARTMAN[1];
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Abstract: 4-Aminopyridine (4-AP) is a general Kv1 (*Shaker*) cytosol-facing channel blocker that prolongs action potentials. Although 4-AP has long been used as a general pharmacological tool to block *Shaker* potassium channels, its clinical use in humans is more recent. 4-AP increases motor activity and is FDA-approved to treat Lambert-Eaton syndrome and ataxia in multiple sclerosis, presumably by improving action potential conduction and increasing neurotransmitter release. Given its relatively non-selective range of voltage-gated potassium channels and pervasive action, it seems a likely candidate for broad and significant side effects. However, literature examining dose-dependent behavioral effects in mammals remains scarce. We aimed to evaluate the dose-dependent effects of orally administered 4-AP on behavior in rats. We gave young male Sprague-Dawley rats (M_{weight} = 219 g + 12 g) *ad libitum* access to a standard diet and water bottles containing water with 0 (control), 0.33 (low), 1.0 (medium), or 3.33 (high) mM 4-AP (n = 12) for 14 days. We assessed several physical and behavioral parameters, including activity levels, sensorimotor coordination, affective behaviors, and neurological fitness before and immediately after the two weeks of treatment. We observed dose-dependent increased grip strength when normalized to body mass, but no other behavioral effects were observed. However, consistent with adverse effects noted in clinical literature, two animals that consumed the high doses demonstrated seizures during the second week of treatment. Our results demonstrated that oral administration of 4-AP in rats increased grip strength relative to mass in a dose-dependent fashion. 4-AP had no significant effect on a number of other behaviors except for the occurrence of seizures in two rats that received among the highest doses. These

data suggest that (consistent with the clinical data) consuming low to medium concentrations of 4-AP may safely improve some neurological functions, but that higher doses (consistent with experimental data) may prolong action potentials to the point of inducing seizures.

Disclosures: A.D. Trofimova: None. **P.S. Gifford:** None. **D.A. Hessinger:** None. **D.L. Bellinger:** None. **R.E. Hartman:** None.

Poster

207. Demyelinating Disorders: Human and Animal Studies and Therapeutics

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 207.11/C80

Topic: B.12. Demyelinating Disorders

Support: National Multiple Sclerosis Society - RG-1701-26750

Title: Targeting OPC-expressed extracellular endosulfatase to overcome the local inhibitory microenvironment following demyelination

Authors: *D. SARASWAT[1], H. J. SHAYYA[1], J. J. POLANCO[2], R. R. WELLIVER[1], S. U. POL[1], J. E. BROOME[1], M. A. O'BARA[1], T. V. KUPPERVELT[3], J. PHILLIPS[4], F. J. SIM[1]; [1]Dept. of Pharmacol. and Toxicology, Jacob's Sch. of Med. and Biomed. Sciences, Univ. at Buffalo, Buffalo, NY; [2]Neurosci. Program, Jacob's Sch. of Med. and Biomed. Sciences,University at Buffalo, Buffalo, NY; [3]Dept. of Biochem., Radboud Inst. for Mol. Life Sciences, Radboud Univ. Med. Ctr., Nijmegen, Netherlands; [4]4Department of Neurolog. Surgery, Univ. of California, San Francisco, CA

Abstract: The inhibitory microenvironment in regions of chronic demyelination acts to prevent adequate activation, recruitment, and differentiation of oligodendrocyte progenitor cells (OPCs) leading to a failure of myelin repair (remyelination) and subsequent axonal atrophy. In a network-based transcriptional analysis, we identified sulfatase 2 (Sulf2) mRNA expression specifically in human primary OPCs and down regulated upon differentiation. Extracellular endosulfatases ,such as Sulf2 ,modulate several cell-cell signaling pathways by modifying 6-O sulfation on heparan sulfate proteoglycans (HSPG) and thereby regulate the accessibility of ligands to their cognate receptors. However, the role of sulfatase in OPC cell dynamics and following demyelination has not been determined. In this study, we found that Sulf2 was catalytically active on and secreted by human OPCs and acted to promote inhibitory Bmp and Wnt signaling. Using lysolecithin-induced spinal cord demyelination, we found that conditional deletion of Sulf1/2 in adult $NG2^+$ OPCs significantly increased the colonization of demyelinated lesions by $Olig2^+$ oligodendrocyte lineage cells and promoted the formation of post-mitotic oligodendrocytes leading to accelerated remyelination. By combining conditional Sulf1/2 deletion with specific manipulation of Wnt and Bmp pathways, we found that sulfatase deletion

in OPCs acts via modulation of both signaling cascades *in vivo* ,promoting a favorable microenvironment for remyelination. Pharmacological inhibition of Sulf1/2 using PI-88, a heparin mimetic, increased HSPG sulfation and relieved inhibitory signaling in cultured hOPCs. Importantly, PI-88 treatment *in vivo* similarly increased oligodendrocyte density during remyelination suggesting the potential for therapeutic intervention in demyelinating disease. Taken together, our findings define an important inhibitory role of OPC-expressed Sulf2 in remyelination and highlight the therapeutic potential for sulfatase inhibition in chronic demyelinating disease.

Disclosures: D. Saraswat: None. **H.J. Shayya:** None. **J.J. Polanco:** None. **R.R. Welliver:** None. **S.U. Pol:** None. **J.E. Broome:** None. **M.A. O'Bara:** None. **T.V. Kuppervelt:** None. **J. Phillips:** None. **F.J. Sim:** None.

Poster

207. Demyelinating Disorders: Human and Animal Studies and Therapeutics

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 207.12/C81

Topic: B.12. Demyelinating Disorders

Support: SFB CRC-128 (B05 to Groppa/Zipp/Meuth)
SFB CRC-128 (B06 to Meuth/Budde/Pape)
DFG (Fa474/5 and BU1019/15-1)

Title: Alterations of neuronal network functionality, brain cytomorphology and behavior following de- and remyelination

Authors: *M. CERINA[1], M. MUTHURAMAN[2], M. GALLUS[1], N. KOIRALA[2], A. DIK[1], L. WACHSMUTH[3], P. HUNDEHEGE[1], P. SCHIFFLER[1], J.-G. TENBERGE[1], V. FLEISCHER[2], G. GONZALEZ-ESCAMILLA[2], V. NARAYANAN[1], C. FABER[3], T. BUDDE[4], S. GROPPA[2], S. MEUTH[1];
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Abstract: Episodes of de- and remyelination, together with immune cells infiltration into the central nervous system (CNS) and inflammation, are recurrent hallmarks in several autoimmune and neurodegenerative diseases like multiple sclerosis (MS). Demyelination is a widely investigated hallmark, however, the degree of damage that this primarily structure alteration induces in the whole brain network activity (as result of white and grey matter regions interaction) as well as on related behavior, is not well understood. To investigate and understand these dynamics, we performed a combined analyses of brain network characteristics obtained

magnetic resonance imaging (MRI with diffusion tensor imaging -DTI), histology and behavioral tests and took advantage of the cuprizone model of general demyelination. Once introduced into the diet of mice, cuprizone induced oligodendrocytes death and therefore significant CNS demyelination, over a period of 5-6 weeks. Indeed, immunohistological evaluation revealed that two weeks of cuprizone diet induced a 52% reduction of myelin content in the corpus callosum (CC, white matter) and a 35% reduction in the neocortex (grey matter). A prolonged diet increased myelin loss in the CC, while remyelination commences in the neocortex. Removal of tis compound from the diet allowed spontaneous remyelination. Anatomical changes are reflected by structural MRI. DTI measurements showed that demyelination is associated with decreased fractional anisotropy (FA) values and increased modularity at the network level. Connectivity alterations involve key anatomical brain regions correlated with impaired memory function and anxiety-like behavior. Interestingly, only for specific networks spontaneous remyelination coincided with amelioration of performance and reconstituted control-like histological values.
Taken together our results support the possibility of identifying novel pathological hallmarks for MS by taking advantage of non-invasive imaging techniques already routinely used in MS diagnosis. Moreover, such hallmarks being associated with specific behaviors and brain connectivity alterations occurring at specific time points of the disease, give the opportunity to identify novel and early windows for diagnosis and paving the way for unravelling the underlying mechanisms.

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Poster

207. Demyelinating Disorders: Human and Animal Studies and Therapeutics

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 207.13/C82

Topic: B.12. Demyelinating Disorders

Title: Characterization of the MOG_{35-55} induced experimental autoimmune encephalomyelitis (EAE) model of MS in mouse by imaging, behavior, gene expression profiling and neurofilament light chain (NfL) quantification

Authors: J. TOIVANEN[1], L. MCMILLAN[2], K. M. PALDANIUS[1], K. LEHTIMÄKI[1], R. NUNAN[2], R. JENKINSON[2], ***J. OKSMAN**[1], D. MISZCZUK[1];
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Abstract: MOG_{35-55} induced experimental autoimmune encephalomyelitis (EAE) in mice is one of the most common preclinical *in vivo* model evoked by inoculation with MOG_{35-55} protein emulsified in complete Freud's adjuvant followed by an injection of pertusis toxin (PTx). The identification of new biomarkers, including gene expression profiling and biofluids based protein is of high interest for the prediction of the disease course and treatment efficacy. In the current study we assess longitudinal disease progression and effect of FTY-720 on clinical manifestation, brain lesion development and immune cell associated gene expression to provide a comprehensive model for efficacy testing.

Mice were inoculated with MOG_{35-55} on D0 and followed until D42. FTY-720, an immune modulating drug approved by FDA for treatment of MS, served as a positive control compound with prophylactic therapy. The daily clinical status of the mice was assessed by clinical scores and body weights. Gadolinium (Gd) contrast enhanced T1-MRI was performed at D14,D 21 and D35 to detect and follow the existence and development of the Gd-enhancing lesions. At D42 cervical segments of spinal cord were collected for nanoString gene expression analysis with an autoimmune profiling panel and neurofilament light chain (NfL) analysis using ultrasensitive Simoa Quanterix system was assessed in CSF.

EAE induced mice start to develop disease symptoms at 8-12 days after immunization, reaching the peak of the symptoms at D17 (mean score 2.6) with complete or partial paralysis of hind limbs accompanied by app. 16% loss of body weight. Disease incidence was 100% by D14. These symptoms were mostly prevented by prophylactic treatment with FTY-720 as 58% of these mice showed no disease onset during the follow-up period. The mean peak clinical score of 0.5 was reached at D19 and no significant body weight loss was observed.

Gd-T1 MRI showed high level of heterogeneity in size, location and time of appearance of Gd enhancing focal lesions, mostly localized in cortex, cerebellum and to a lesser extent in striatum. FTY-720 seemed to delay the lesion appearance, but did not prevent them completely. In addition, many of the EAE animals showed widespread diffuse Gd-enhancement that may be related to PTx modulation of BBB permeability. Moreover, changes in immune gene expression profiles in spinal cord and NfL levels in CSF provide evidence for ongoing axonal damage in response to EAE.

Our findings underscore the value of a holistic phenotyping strategy in mouse MOG_{35-55} EAE model as a useful tool to examine molecular mechanisms and therapeutic targets in detail, and to study the efficacy of therapeutics against MS.

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Poster

207. Demyelinating Disorders: Human and Animal Studies and Therapeutics

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 207.14/C83

Topic: B.12. Demyelinating Disorders

Support: ISF grant 0399306
Grant by the Ministry of Science, Technology and Space, Israel 3-13395

Title: Predicting conduction delays using structural MRI in multiple sclerosis patients

Authors: *S. BERMAN[1], D. KARUSSIS[2], N. LEVIN[2], Y. BACKNER[2], P. PETROU[2], A. A. MEZER[3];
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Abstract: In recent years white matter tissue is being regarded as a dynamic tissue that undergoes activity-dependent structural changes (Fields, 2015). The White matter consists mainly of myelinated and non-myelinated axons, forming networks which are necessary for cognitive functions (Moeller et al., 2015), normal development and normal aging (Nagy et al., 2004; Yeatman et al., 2014). Technological developments in quantitative MRI (qMRI) techniques make it possible to measure a variety of white matter microstructure properties in humans *in vivo* (Wozniak & Lim, 2006). However it is still unclear whether these measurements of white matter microstructure can allow modeling of the variance in the white matter's main functional role - signal conduction.
Our recent work developed a framework that uses qMRI measurements of white matter microstructure to model estimates of conduction delays along white matter fibers (Berman et al., 2019). Using myelin-sensitive as well as diffusion-sensitive measurements the framework provided conduction estimates which are in agreement with the literature. To test whether qMRI can explain the variance in conduction estimates, we tested our hypothesis on patients diagnosed with multiple sclerosis (MS), which are expected to have high variance in both structure and function of white matter. MS is a chronic demyelinating disease of the central nervous system, in which myelin is attacked, changing white matter structure and leaving lesions (Compston & Coles, 2008). The damage to white matter in MS affects the signal conduction (Halliday et al., 1973). We use qMRI to measure microstructure properties of the optic radiation and optic tract of these subjects, as well as visual evoked potentials (VEP) to assess the conduction latency along the same tracts. Using a cross-validation approach we are able to predict the VEP latency using the structure of the white matter tract of the visual pathway ($p<10^{-5}$).
This work connects the functional variation in human white matter as expressed in conduction along white matter tracts, and the structural variation along these tracts. Applying these methods in MS patients with white matter abnormalities is essential for filling the prominent gap in understanding the outcomes of changes to white matter structure and function in humans *in vivo*.

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Poster

207. Demyelinating Disorders: Human and Animal Studies and Therapeutics

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 207.15/C84

Topic: B.12. Demyelinating Disorders

Support: The Mayo Clinic Center for MS and Autoimmune Neurology
The Mayo Clinic Center for Biomedical Discovery
The MN State SCI and TBI Research Program
the Neilsen Foundation

Title: Chronic high fat diet results in mitochondria dysfunction and oligodendrocyte loss in the spinal cord in a NAD-dependent fashion

Authors: *M. R. LANGLEY[1], H.-S. YOON[2], H. N. KIM[1], W. SIMON[1], L. KLEPPE[1], I. R. LANZA[3], A. MATVEYENKO[3], N. LABRASSEUR[4], C. CHINI[5], E. CHINI[6], I. A. SCARISBRICK[2];
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Abstract: Metabolic syndrome is a risk factor and co-morbidity in multiple sclerosis (MS), so a better understanding of how a high fat diet (HFD) contributes to oligodendrocyte loss has the potential to highlight new therapies. In 10 wk old C57Bl/6 male mice fed a 60% kcal HFD, we found significant decreases in vertical activity by 4 wk and significant changes in ambulatory and stereotypy movements by 12 wk in the open field test. HFD-fed mice also spent less time in the center of the field as opposed to the perimeter, indicating an anxious phenotype. Mice fed a HFD for 12 wks had a significant loss of oligodendrocytes and increased signs of lipid peroxidation (4-Hydroxynonenal immunoreactivity) and apoptosis in white matter of the brain and spinal cord. Alterations in mitochondria function, TCA cycle metabolites, and NAD metabolism were also observed in the spinal cords of HFD-fed mice. In cell culture, when exposed to a saturated fat (palmitate, PA, 100µM) for 24 hrs, oligodendrocytes display decreased mitochondrial function, altered mitochondrial morphology, and impaired differentiation. However, oligodendrocyte differentiation is improved upon addition of exogenous NAD to the PA-exposed cultures. Similar effects are seen indirectly by treating oligodendrocytes with conditioned medium obtained from astrocytes cultured with PA. Together, these findings suggest that a change in the spinal cord in response to consumption of a HFD creates an environment less

conducive to neural repair processes in neurological disorders by impairing mitochondria function and thus oligodendrocyte survival. Moreover, pharmacological inhibition of CD38, a NAD+-degrading enzyme, with 78c can overcome the dysmyelinating effects of lysolecithin and PA in *ex vivo* organotypic cerebellar slices and pro-inflammatory responses in astrocytes, revealing an immense translational value of this target for MS treatment.

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Poster

207. Demyelinating Disorders: Human and Animal Studies and Therapeutics

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 207.16/C85

Topic: B.12. Demyelinating Disorders

Support: NMSS RG4257B4/1
NIH RO1 NS036647
NIH Training Grant T32ES007148

Title: The metabotropic receptor agonist, CHPG, reverses clinical signs in mice subjected to experimental autoimmune encephalomyelitis (EAE)

Authors: *T. M. PLANAS FONTANEZ[1], A. DE STEFANO[3], T. SULLIVAN[4], Y. HUANG[2], C. F. DREYFUS[5];
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Abstract: Most treatments for demyelinating diseases, such as Multiple Sclerosis, focus on reduction of the immune response. However, a complementary issue is how to prevent or reverse demyelination, itself. Previous studies in our laboratory demonstrated that a metabotropic glutamate receptor (mGluR) agonist, 1-amino-1,3-dicarboxycyclopentane (ACPD), reverses deficits in brain-derived neurotrophic factor (BDNF) and myelin protein levels following injury in a cuprizone model of demyelination. Group I mGluRs (mGluR-1 and mGluR-5) were localized to astrocytes and effects of ACPD were found to be dependent upon the production of BDNF by these cells (Fulmer et al, 2014). Recent work indicates that similar effects are elicited by an intraperitoneal (ip) injection of the Group I mGluR agonist, 2-chloro-5-hydroxyphenylglycine (CHPG; Saitta and Dreyfus, personal communication). In this study, we tested whether CHPG could reverse clinical signs in EAE mice immunized with myelin oligodendrocyte glycoprotein (MOG). This demyelinating model exhibits an immune response as well as a neural response. The EAE clinical scores were assessed for each animal according to

the following criteria: 0=no signs of disease, 1=loss of tail tone, 2=moderate hind limb paralysis, 3=complete hind limb paralysis, 4=complete forelimb paralysis, and 5=moribund or dead. Ip injections of CHPG (20 mg/kg, injected every other day) delayed MOG-induced EAE, and ameliorated clinical signs when treatment was initiated after mice developed hindlimb paralysis. This effect was accompanied by reversal of the loss in BDNF and myelin proteins in the lumbar spinal cord that followed initiation of EAE. Moreover, increased co-localization of mGluR-5 with GFAP+ astrocytes was noted within lesion sites in preliminary work, with rare co-localization with Iba1+ activated microglia, or CD11b+ microglia and macrophages, suggesting that astrocytes or microglia may be targets of CHPG action. In contrast, no co-localization of the receptor with CD4+ T-helper cells, CD45R+ B-cells, or Ly-6g+ neutrophils was observed, indicating that peripheral immune cells do not appear to express mGluR-5 at early or late stages of disease. Future studies will be performed to define cellular mechanisms underlying the effects of CHPG on glial cells and continue to explore the potential of metabotropic agonists as targets for treating demyelinating diseases. Supp. NMSS RG4257B4/1, NIH RO1 NS036647 and NIH Training Grant T32ES007148.

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Poster

207. Demyelinating Disorders: Human and Animal Studies and Therapeutics

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 207.17/C86

Topic: B.12. Demyelinating Disorders

Title: Refinement of axonal function during development of the mouse optic nerve

Authors: *A. BALRAJ, R. H. MILLER;
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Abstract: The optic nerve is a white matter tract and in the adult, virtually all the axons are myelinated. Previous studies have shown that in the mouse optic nerve, myelination of retinal ganglion cell axons begins around postnatal day 7 and continues until the fifth week. During this period, the nerve increases in cross-sectional area as RGC projections are established. While the nerve is considered morphologically stable beyond 5 weeks of age (wks), nerve function has yet to be characterized. This study evaluates optic nerve conduction capacity in C57BL6 mice (aged 4-12 weeks) using *ex vivo* electrophysiological recordings of compound action potentials (CAPs). CAPs were induced by an electrical stimulus of varied intensity (7-80V), where increased voltage recruits additional axons. The waveform at maximal intensity represents the relative number of responsive axons in a nerve (as area under the curve) and axon populations with distinct conduction speeds (as peaks). To assess differences in axon population

characteristics, each peak was plotted by latency and area, and a K-means cluster analysis was used to determine differences between populations across age. The average CAP area increases between 4 and 5 wks as expected, but continues to vary with age. When pooled, axon populations across all ages favor faster conduction and cluster into four distinct groups. Responses from all ages are equally represented in the three fastest groups. However, the slowest axon population group is specific to younger ages (4-6 wks), suggesting that slow populations are lost as the optic nerve matures. Analysis of 5 week and 8 week old optic nerves by electron microscopy demonstrate a shift to large diameter axons with age consistent with our functional assessment. We conclude that these changes in optic nerve function after initial development reflect an additional period of refinement which may be a consequence of altered myelination or axonal loss.

Disclosures: A. Balraj: None. **R.H. Miller:** None.

Poster

207. Demyelinating Disorders: Human and Animal Studies and Therapeutics

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 207.18/C87

Topic: B.12. Demyelinating Disorders

Support: NMSS Collaborative Research Center
NMSS Research Grant
Guthy Jackson Charitable Organization
NIH R01EY022936
NIH UM1AI110498

Title: Absence of microglial activation inhibits complete remyelination in cerebellar slices

Authors: K. S. GIVEN[1], Y. LIU[2], G. P. OWENS[2], J. L. BENNETT[2], ***W. B. MACKLIN**[1]; [1]Cell and Developmental Biol., [2]Neurol., Univ. of Colorado:AMC, Aurora, CO

Abstract: Remyelination is the process by which new myelin sheaths are generated around axons following pathological loss of myelin. Accumulating evidence suggests that microglia play an important role in this process through removal of debris and promotion of oligodendrocyte differentiation. We are investigating the role of microglia during remyelination following inflammatory demyelination caused by recombinant antibodies cloned from multiple sclerosis patients. In our cerebellar explant model, two distinct peaks of microglial activation are observed: the first occurs at the end of demyelination (T0), while the second, more pronounced peak, occurs 3 days later (T3) during remyelination which coincidentally marks the return of mature oligodendrocytes within the folia. When microglia are pharmacologically depleted during remyelination using PLX5622, the maturation of oligodendrocytes to the myelinating stage is

impaired, as is the subsequent remyelination of denuded axons. In another paradigm, microglial activation is reduced by the presence of excess myelin binding antibody in the media, which also leads to impaired oligodendrocyte maturation and incomplete remyelination. Importantly, we have identified specific populations of microglia using scRNAseq during remyelination and under inhibitory conditions. These data suggest that microglia play a significant role in oligodendrocyte maturation and remyelination. Our studies provide insight into a distinct activated microglia population that promotes and apparently is needed for oligodendrocyte maturation during remyelination after an inflammatory injury such as those found in the pathology of multiple sclerosis.

Disclosures: K.S. Given: None. **Y. Liu:** None. **G.P. Owens:** None. **J.L. Bennett:** None. **W.B. Macklin:** None.

Poster

207. Demyelinating Disorders: Human and Animal Studies and Therapeutics

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 207.19/C88

Topic: B.12. Demyelinating Disorders

Title: Cns pathology and motor deficits observed following injection of multiple sclerosis CSF derived recombinant antibodies is limited to primary progressive MS

Authors: F. CALI, A. L. TSE, J. K. WONG, *J. LIN, S. A. SADIQ;
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Abstract: The use of oligoclonal bands found in the cerebrospinal fluid (CSF) of multiple sclerosis (MS) patients as a diagnostic marker and the widespread use of anti-B cell treatments suggest an important role of B-cells in MS. Our previous studies have shown that intrathecal injections in mice with CSF from primary progressive multiple sclerosis (PPMS) patients result in motor deficits, astrogliosis, and axonal damage compared to control mice injected with saline or recombinant antibodies (rAbs) isolated from the CSF of patients with other neurological disorders. Aiming to pinpoint the CSF component causing the observed motor deficits and CNS pathology, our study focuses on the effect of intrathecal injections of rAbs produced from each subtype of MS patient CSF.
MS CSF samples were obtained by standard lumbar puncture and immediately analyzed by fluorescence-activated cell sorting (FACS) for $CD19^+$ and/or $CD138^+$ B-cells. Full IgG1 rAbs were produced. Mice underwent laminectomies at cervical levels 4 and 5 and CSF rAbs were injected under the dura mater into the subarachnoid space. In total 101 female mice received intrathecal injections of rAbs or saline, with non-MS control antibodies isolated from HTLV-1 and ALS patient CSF. 12 PPMS, 4 RRMS, 4 SPMS and 3 non-MS antibodies were injected. Motor deficits were defined through assessment of forelimb gripping and reaching, tail rigidity

and using a grip strength meter. Mice were perfused at one-day post injection for histological analysis. All motor testing and histology was performed blinded.
Results showed significant differences in motor deficits between mice injected with rAbs from each MS subtype. 74% of mice injected with PPMS rAbs showed motor deficits one day after injection, significantly higher than all other groups. There was no significant difference between motor deficits observed in mice injected with RRMS, SPMS, non-MS control rAbs or saline. Immunostaining supported the observed motor changes. Luxol fast blue staining revealed demyelination in 3 mice injected with 3 different PPMS rAbs that also displayed motor deficits, but in no mice injected with RRMS, SPMS, non-MS rAbs or saline. Anti-human IgG immunostaining was detected following some PPMS rAb injections. Significantly higher intensity GFAP and SMI-32 staining in PPMS rAb injected mice suggest astrogliosis and axonal damage.
Antibodies derived from PPMS CSF likely contribute to motor deficits and CNS pathology in mice. Future investigations will aim t to elucidate the pathogenic role and potential target of these CSF antibodies in MS.

Disclosures: F. Cali: None. **A.L. Tse:** None. **J.K. Wong:** None. **J. Lin:** None. **S.A. Sadiq:** None.

Poster

207. Demyelinating Disorders: Human and Animal Studies and Therapeutics

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 207.20/C89

Topic: B.12. Demyelinating Disorders

Support: Tisch MS Research Center (private funds)

Title: Cerebrospinal fluid pheresis may have therapeutic value in primary progressive multiple sclerosis

Authors: *J. K. WONG[1], A. K. ROSELLE[1], S. J. E. SHIMSHAK[1], L. ZITELLA VERBICK[2], N. NAZARIAN[2], A. MCCABE[2], S. A. SADIQ[1];
[1]Tisch MS Res. Ctr. of New York, New York, NY; [2]Minnetronix Neuro, Saint Paul, MN

Abstract: Multiple sclerosis (MS) is characterized by inflammatory demyelination, astrogliosis and axonal loss in the CNS. Approximately 10-15% of MS patients are diagnosed with primary progressive multiple sclerosis (PPMS), which is characterized by unremitting disease progression from disease onset. We have previously reported that intrathecal delivery of PPMS cerebrospinal fluid (CSF) induces motor deficits and spinal cord pathology in mice. Here, we investigated whether filtration of PPMS CSF would remove pathology-inducing factors and improve functional outcome in mice. A tangential flow filtration (TFF) system was used to pass

PPMS CSF through 5kDa hollow-fiber filters. Following 3 filtration cycles, CSF protein concentration was significantly reduced, as measured by BCA assay. 8-10 week old female mice underwent laminectomies at cervical levels 4 and 5, and either PPMS CSF or filtered PPMS CSF was injected under the dura mater into the subarachnoid space. Control animals were injected with saline. All motor testing and histological analyses were performed in a blinded manner. Functional deficits were assessed by evaluating forelimb grip strength, reaching accuracy and tail rigidity at 1 day following intrathecal CSF delivery. Mice injected with PPMS CSF displayed significantly higher motor deficit scores and weaker grip strength than mice injected with filtered PPMS CSF and saline controls. Mice were perfused at 1 day post injection (DPI). Spinal cords were post-fixed overnight in 4% paraformaldehyde, cryoprotected in 30% sucrose, then cryosectioned for histological analyses. In cervical spinal cords of PPMS CSF-injected mice, demyelinated lesions were observed, as revealed by luxol fast blue staining. At 1DPI, evidence of reactive astrogliosis and axonal damage was also observed, as revealed by significantly stronger GFAP and SMI-32 immunostaining intensities, respectively. None of these pathological changes were observed in mice injected with filtered PPMS CSF, or control mice. These data provide preclinical support for the testing of Neurapheresis^{TM} Therapy in PPMS patients.

Disclosures: **J.K. Wong:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Minnetronix Neuro. **A.K. Roselle:** None. **S.J.E. Shimshak:** None. **L. Zitella Verbick:** A. Employment/Salary (full or part-time):; Minnetronix Neuro. **N. Nazarian:** A. Employment/Salary (full or part-time):; Minnetronix Neuro. **A. McCabe:** A. Employment/Salary (full or part-time):; Minnetronix Neuro. **S.A. Sadiq:** None.

Poster

207. Demyelinating Disorders: Human and Animal Studies and Therapeutics

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 207.21/C90

Topic: B.12. Demyelinating Disorders

Title: Measuring high field MRI imaging pipeline specificity using novel mouse brain model with diverse lesions

Authors: *S. U. POL[1], J. BONSIGNORE[2], M. DWYER[2], M. PREDA[2], M. SVEINSSON[2], F. SCHWESER[2], R. ZIVADINOV[2];
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Abstract: Clinician's guidelines for selecting a suitable MS therapy rely on reproducible MRI measures such as T2 lesion load. Unfortunately, clinicians cannot determine the changes in myelin and inflammatory status of MRI visible lesions. Hence, emerging multimodal imaging tools capable of closely tracking disease response are better suited for therapy selection. However, there is a lack of pre-clinical models to test and validate such tools. Currently

employed demyelination models were primarily designed for studying MS therapies or myelin biology and not for testing imaging tools. Therefore, we aimed to generate a pre-clinical model that can concomitantly present lesions with diverse pathologies. For this, we stereotactically injected lysolecithin into the two internal capsules (IC)s separately (female mice 6-7 weeks of age, C57/BL6). The injections were conducted 17 days apart. We hypothesized that at 23 days post first injection (dPI), the two lesions will be at different stages of repair.
First using dye injections, we confirmed that stereotactic coordinates (Anterior-Posterior = 0.95, Medial-lateral 3.55, Dorsal-ventral = 2.9), needle approach angle (25° to vertical) and injection volume (5µl) were optimal parameters for targeting ICs. We noted that ICs with 5dPI lesions exhibited significantly higher density of Iba1 positive microglia relative to ICs with no lesions or 23dPI lesions. Additionally, we noted no changes in Olig2 expressing oligodendrocyte density in 5pfi lesioned IC vs no lesion IC. However, we noted that there was a significant decrease in $CC1^{+}/OLIG2^{+}$% differentiated oligodendrocyte in ICs with 5 day old lesions. Next we assessed the myelin levels in the ICs using Eriochrome and fluoromyelin staining. We noted that lesioned ICs had a significantly lower Eriochrome labelled myelin relative to healthy control animals. Additionally, 23dPI lesions exhibited higher amount of Eriochrome staining relative to 5pfi lesions. These areas of demyelination were visible in the *ex vivo* MRI scans in the matching locations.
We could show that lysolecithin lesions generated at different time points prior to imaging timepoints produce lesions with differing myelin and inflammatory status. The lesioned brains can be scanned with a combination of MRI imaging modalities such as DTI and QSM. This will allow us to generate a head to head comparison and optimal combination of test imaging modalities.

Disclosures: **S.U. Pol:** None. **J. Bonsignore:** None. **M. Dwyer:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Novartis and Celgene. F. Consulting Fees (e.g., advisory boards); Claret Medical and EMD Serrono. **M. Preda:** None. **M. Sveinsson:** None. **F. Schweser:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); SynchroPet. F. Consulting Fees (e.g., advisory boards); Toshiba Canada Medical Systems ltd, Goodwin Procter LLP. **R. Zivadinov:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Genzyme-Sanofi, Novartis, Celgene, Claret Medical, Intekrin-Corherus, Oritembis and Quintiles/IMS. F. Consulting Fees (e.g., advisory boards); EMD Serono, Genzyme-Sanofi, Claret Medical, Celgene and Novartis.

Poster

208. Brain Wellness and Aging: Pharmacological and Non-Pharmacological Interventions

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 208.01/C91

Topic: C.01. Brain Wellness and Aging

Support: NIH 1T32AG057468-01
NIH 5RO1HL129153-03
UIC Provost Deiss Award
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UH3 NS100127
UIC Chancellor's Proof-of-Concept-Award

Title: The potential of plasma lipoprotein profiles as a cognitive biomarker in a dietary intervention for obese females

Authors: *A. KARSTENS[1,2], S. CORONEL[2], K. PANCHAPAKESAN[2], Y. SALEH[2], B. XIANG[2], A. C. VALENCIA[2], M. LADU[2];
[1]Psychology, [2]Anat. and Cell. Biol., Univ. of Illinois, Chicago, Chicago, IL

Abstract: Alzheimer's disease (AD) is the most common neurodegenerative disorder in older adults. With no cure and few palliative treatments, non-pharmacological interventions (specifically, the Mediterranean Diet/MedDiet) that target modifiable risk factors are increasingly being investigated. While age is the greatest risk factor for AD, the greatest genetic risk factor is *APOE4*, increasing risk 4- to 12-fold compared to the common *APOE3*. *APOE4* risk is increased for female carriers and exacerbated further by modifiable risk factors including obesity, poor diet, and commonly co-morbid dyslipidemia. *APOE* encodes apolipoprotein E (apoE), a protein component of lipoproteins and the major apolipoprotein expressed in the brain. Separate from the brain, plasma lipoproteins in the blood are heterogenous, including very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL). Traditional blood lipid panels report total lipid levels and lipid levels associated with isolated lipoproteins, with an emphasis on HDL/LDL cholesterol. In contrast, plasma lipoprotein profiles generated via fast liquid protein chromatography (FPLC) are modulated by age-, *APOE*-genotype and sex. For example, *APOE4* carriers have more dyslipidemic profiles (>VLDL/LDL, <HDL) relative to *APOE3* carriers. In addition, FPLC elutes intact lipoproteins from largest to smallest (chylomicrons/VLDL, LDL, HDL-2, HDL-3, and free proteins) with fractions that can be further analyzed for lipid/protein content (e.g., cholesterol, apoE). It is unclear whether diet can modify the dyslipidemic plasma lipoprotein profile in female *APOE4* carriers, and, in turn, improve cognition. We hypothesize a rightward, normolipidemic shift (<VLDL/LDL; >HDL) in the plasma lipoprotein profile with the MedDiet, particularly in the *APOE4*, carriers. This study leverages pre- and post-intervention data from an 8 month randomized controlled trial of the MedDiet in obese female older adults (≥ 55 years) to improve cognition. FPLC was used to analyze fasting blood plasma at baseline and post-intervention. We present preliminary data, blind to group, to demonstrate predicted group status (MedDiet vs. Usual Diet) based on plasma lipoprotein profile shifts from baseline to post-intervention. We specifically hypothesize that normolipidemic shifts in the plasma lipoprotein profile reflect the MedDiet group whereas dyslipidemic shifts reflect the usual diet control. If our hypothesis is correct, this work would support the use of diet interventions to modify risk in *APOE4* females and plasma lipoprotein profiles as a biomarker to monitor cognitive decline.

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Poster

208. Brain Wellness and Aging: Pharmacological and Non-Pharmacological Interventions

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 208.02/C92

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant MH109466-03
NIH Grant MH109466-04S

Title: Histone deacetylase inhibitor tacedinaline decreases aged related neurological side effects induced by haloperidol

Authors: ***B. M. MCCLARTY**, G. RODRIGUEZ, S. CHAKRABORTY, H. DONG; Psychiatry and Behavioral Sci., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: Elderly patients, particularly in Alzheimer's disease with the behavioral and psychological symptoms of dementia (BPSD) are commonly treated with antipsychotic medications. However, antipsychotics induced extrapyramidal side effects (EPS) are much higher in elderly patients compared to younger ones. Primary literature and our laboratory have shown an age-related reduction in the primary target of typical antipsychotics, the dopamine 2 receptor (D2R) in the brain that may increase the sensitivity of the side effects. It is thought that histone modifications could regulate D2R expression and functioning in aging thereby modulating drug action. Our preliminary data has shown class 1 histone deacetylase (HDACs), especially HDAC1 and 3 increased in the aged striatum, suggesting class 1 HDACs may play a significant role in D2R function. In this study, we tested a specific HDAC1 and 3 inhibitor, Tacedinaline (CI-994), potential role to reduce the motor side effects induced by haloperidol (HAL), a typical antipsychotic in aged mice, in a dose dependent manner. We intraperitoneal (IP) injected HAL in C57BL/6 mice at 20-months of age with either vehicle, HAL (0.05 mg/kg), CI-994+HAL or CI-994 (10, 20 or 30 mg/kg) for 4 (acute) and 14 (chronic) consecutive days. Then mice were subjected to 3 motor behavioral tasks including open field, rotarod and catalepsy. After behavior testing, striatal and cortical tissues were extracted for biochemical analysis. CI-994 at a dosage of 30 mg/kg displayed side effects itself including respiratory problems and severe sedation, therefore we excluded the dose of 30mg/kg in this study. Our results showed that CI-994 at doses of 10 and 20 mg/kg significantly decrease HAL induced ESP-like side effects measured by catalepsy either in 4 days or 14 days ($p<0.0001$) in CI-994+HAL groups as compared to HAL alone and increased the motor function measured in rotarod test either in 4 days or 14 days ($p<0.05$) for 20 mg/kg treatment, but not at 10 mg/kg. Trends of increased motor function in the open field test were displayed in both 10 and 20 mg/kg

doses. Moreover, CI-994 at a dose of 20 mg/kg for 14 days displayed more robust beneficial effects in motor function as compared to 10 mg/kg dose groups. Our results suggested a dosage of 20 mg/kg CI-994 in conjunction with 0.05 mg/kg of HAL can reduce the severity of EPS-like side effects and improve motor function in aged C57BL/6 mice and is the most efficient for chronic treatment. To reveal the underlying mechanisms of the behavioral phenotypes, next we will investigate the D2R expression and functioning and the histone modifications at the D2R promotor in the striatum in response to CI-994 and HAL administration.

Disclosures: B.M. McClarty: None. **G. Rodriguez:** None. **S. Chakraborty:** None. **H. Dong:** None.

Poster

208. Brain Wellness and Aging: Pharmacological and Non-Pharmacological Interventions

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 208.03/D1

Topic: C.01. Brain Wellness and Aging

Support: CMHS UAE University Grant 31M201
CMHS UAE University Grant 31M102

Title: Effects of transcranial direct current stimulation of the dorsolateral prefrontal and parietal cortex on dual-task performance involving manual dexterity and cognitive task in healthy old adults

Authors: M. R. LJUBISAVLJEVIC[1], J. OOMMEN[1], S. FILIPOVIC[2], J. BJEKIC[2], N. SZOLICS[3], N. NAGELKERKE[4], *G. C. BAYLIS[5];
[1]Col. of Med. and Hlth. Sci., Al Ain, United Arab Emirates; [2]Inst. for Med. Res., Belgrade, Serbia; [3]Tawam Hosp., Al Ain, United Arab Emirates; [4]Col. of Medicien and Hlth. Sci., Al Ain, United Arab Emirates; [5]United Arab Emirates Univ., Al Ain, United Arab Emirates

Abstract: Performing two tasks simultaneously often causes a decline in one or both tasks. The interference when performing dual-task is believed to be caused by competition of shared brain regions that are needed for both tasks (structural interference). The so-called dual-task costs are more pronounced in old than in young adults. In old adults, structural interference may be related to the reduced residual capacity of the brain regions so that the cognitive resources involved in processing become exhausted. Also, there is increasing evidence that aging is associated with an expansion of brain activation, which may also result in higher dual-task interference in older adults. This study aimed to examine whether modulation of cortical activity through the non-invasive brain stimulation technique, transcranial direct current stimulation (tDCS), could enhance the performance of demanding motor-cognitive dual-task in healthy old adults. Here we report preliminary results based on ten healthy old age volunteers who underwent real or sham

anodal F4 - cathodal P4 cortical tDCS while performing the grooved pegboard test (GPT) and serial seven subtraction test (SSST) alone or together (dual-tasking) in a cross-over, single-blinded, randomized design. The number of pegs and the number of correct subtractions were recorded before, during and 30 minutes after tDCS (20 minutes @ 1.5 mA). The dual-task performance was measured as the percent change from single to the dual-task condition (dual-task cost - DTC). We hypothesized that the anodal (excitatory) prefrontal (F4) combined with cathodal (inhibitory) stimulation of parietal cortex (P4) would significantly reduce the interference of both tasks. The preliminary results show that neither real nor sham tDCS altered performance on the single-task. Dual-task performance during tDCS for GPT was -16.6% (tDCSreal) versus -17.3% (tDCSsham) and for SSST was -19.5% (tDCSreal) versus -24.2% (tDCSsham). Against our hypothesis, the difference between real and sham tDCS was not significant although there was a trend for real tDCS to improve serial subtraction performance. The larger sample size is needed to draw further conclusions about the potential use of prefrontal-parietal tDCS approach to improving cognitive and/or motor performance in daily living by enhancing the system's capacity to adapt to stressors in old adults.

Disclosures: G.C. Baylis: None. **M.R. Ljubisavljevic:** None. **J. Oommen:** None. **S. Filipovic:** None. **J. Bjekic:** None. **N. Szolics:** None. **N. Nagelkerke:** None.

Poster

208. Brain Wellness and Aging: Pharmacological and Non-Pharmacological Interventions

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 208.04/D2

Topic: C.01. Brain Wellness and Aging

Support: MOTIE; No.10067221

Title: Light driven neurovascular modulation as a novel treatment method for mild cognitive impairment

Authors: *W.-C. JEONG[1], N.-G. KIM[1], C. HEO[2], K.-J. PARK[1], J. LEE[1], M.-H. RYU[3], J. KIM[4], K. PAK[5], Y.-H. KIM[6], Y.-I. SHIN[7];
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Abstract: Reduced cerebral blood flow (CBF) is an early biomarker for neurodegenerative diseases such as mild cognitive impairment (MCI), Alzheimer’s disease (AD), and dementia, and its alleviation is key to prevention of disease progression. However, lack of a successful

treatment for increasing CBF flow underlines the need for new effective and safe treatments. Here we propose an extracranial light stimulation (ELS) method (610 nm of peak wavelength, 3.0 mW/cm^2, 30 min). a new technique for MCI treatment, which targeted spots on the neck skin over carotid and/or vertebral arteries. In a pilot clinical trial, light was administered for 4 weeks (20 times/once per day) in patients with MCI (n=22) and healthy subjects (n=24). Neurological assessments indicated that the long-term ELS treatment improved memory, depression and general cognition in MCI patients, while only memory was enhanced in healthy subjects. Extracranial light irradiation increased regional CBF measured by single photon emission tomography and the amplitude of oxyhemoglobin concentration signals measured by functional near-infrared spectroscopy in the multiple cortical regions including the prefrontal, frontal, temporal and occipital areas in healthy subjects. Additionally, we demonstrated that the acute ELS dilated cortical blood vessels (< 10 μm) of the wild-type mice, but not of the neuronal nitric oxide synthase (nNOS) knockout mice using two-photon microscopy. Our findings suggest that the photons emitted from a skin-adhesive light irradiation unit may stimulate nerve endings beneath the skin, transmitting this signal to induce secretion of neuronal NO from nitrergic nerve terminals. This NO would participate in the dilation of capillaries in the brain leading to increase in local blood flow, so that it will enhance the supply of oxygen and nutrients to the compromised neurons, which could result in improving cognition. Our results from the clinical and preclinical studies indicate that an extracranial light stimulation method has potential as a new effective and safe non-pharmaceutical method for improving CBF and cognitive function in neurological disorders.

Disclosures: **W. Jeong:** A. Employment/Salary (full or part-time):; Color Seven Co., Ltd. **N. Kim:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Color Seven Co., Ltd. **C. Heo:** None. **K. Park:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Color Seven Co., Ltd. **J. Lee:** A. Employment/Salary (full or part-time):; Color Seven Co., Ltd. **M. Ryu:** None. **J. Kim:** None. **K. Pak:** None. **Y. Kim:** None. **Y. Shin:** None.

Poster

208. Brain Wellness and Aging: Pharmacological and Non-Pharmacological Interventions

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 208.05/D3

Topic: C.01. Brain Wellness and Aging

Support: Spanish Ministry of Economy and Competitiveness (PSI2017-84933)

Title: Physical activity context correlates with hippocampal subfield and nuclei of the amygdala volume

Authors: *S. LOZANO-SEOANE, L. EZAMA-FORONDA, N. JANSSEN;
Univ. of La Laguna, San Cristóbal de La Laguna, Spain

Abstract: Many studies have shown that certain physical activity (PA) produces changes in the hippocampus that correlate with improved cognitive performance. However, current studies have not examined the association between PA performed in different habitual contexts and volume of the hippocampal subfields and the nuclei of the amygdala. In the present study, we inspected this issue using Surface Based Morphometry in Magnetic Resonance Imaging data. 30 participants, mean age 22.57 years, 51.61% male from the Canary Islands, Spain, took part in the experiment. Their regular PA was assessed through a questionnaire which categorizes PA in three different contexts and constructs: Work index (WI), that refers to the occupational PA; Sports index (SI), sport during leisure time; and Leisure-time index (LI), leisure-time activities other than playing sports. MRI preprocessing and automated segmentation of hippocampal subregions and amygdala nuclei were performed using FreeSurfer v6.0.0. We analyzed the data for each hippocampal structure and amygdala nucleus with multiple regression analyses. Variables included in the model were subfield or nuclei volume, WI, SI, LI, gender, estimated total intracranial volume (eTIV), body mass index (BMI) and hemisphere. Our results showed that WI was a good predictor of CA4 and DG in the hippocampus and of basal (Ba), accessory basal (AB), medial (Me) and Cortical (Co) nuclei of the amygdala. SI was a good predictor of CA2/3 in the hippocampus, showing a negative association. LI was a good predictor of Paralaminar (PL) and Anterior (AAA) nuclei of the amygdala volumes. Gender was associated with Me and Co. Also, eTIV showed a positive association with every subfield and nucleus volume except for Me and Co. BMI had a positive relationship with volumes of CA2/3, CA4 and DG of the hippocampus and with AB, Co and the corticoamygdaloid transition area of the amygdala. Finally, hemisphere was a good predictor for each hippocampal subfield and nucleus volume, except for Me and PL. Variance Inflation Factor was calculated for each model with no collinearity identified. Extending previous studies, our results suggest that increased PA during work increased CA4, DG, Ba, AB, Me and Co volume. In addition, increased PA during leisure time increased PL and AAA volume. Surprisingly, increased PA during sports was associated with decreased CA2/3 volume. This contrast may suggest different impacts of PA contexts on these substructures and are relevant for designing PA programs in therapeutic interventions.

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Poster

208. Brain Wellness and Aging: Pharmacological and Non-Pharmacological Interventions

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 208.06/D4

Topic: C.01. Brain Wellness and Aging

Support: National Natural Science Foundation (31871143)

Title: Dorsolateral prefrontal cortex plasiticiy based video game training in older adults

Authors: *H. LI[1], P. WANG[2];
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Abstract: Previous studies consistently found age-related brain structural and functional disruptions in dorsolateral prefrontal cortex (DLPFC). DLPFC plays important roles in higher cognitive functions including working memory, inhibition, episodic memory, decision-making, reasoning, problem solving and attention. In the current study, through custom-designed video game training, we aimed to explore the DLPFC structural and functional plasticity in older adults, as well as the DLPFC-related cognitive functions. Seventy older participants were recruited and randomly assigned into experimental group and active control group. All participants completed 12 1-hour training sessions in the laboratory over a period of 6 weeks. The training game for experimental group was a game combined with fruit slicing game and N-back task ranging from 0-back to 3-back; while the game for active control group was designed only according to fruit slicing game and 0-back task. Cognitive assessments in different cognitive domains, including working memory, visuospatial ability, attention, episodic memory and processing speed were conducted on participants in pre-test and post-test. Structural magnetic resonance imaging (MRI) scans and resting state functional MRI scans were conducted before and after the training. Compared to the control group, participants in the experimental group showed an enhanced trend in work memory ability. The training-induced improvement in working memory predicted the gray matter volume increase in DLPFC. Furthermore, regional homogeneity in DLPFC of experimental group also increased. The DLPFC-based video game training induced extended benefits to untrained visuospatial processing, attention and episodic memory. The present study revealed that DLPFC based working memory training game was effective to improve cognitive and brain functions in older adults. The current study, originated from aging neuroscience findings and specially designed for older adults with cognitive psychology paradigm, provided a direction for future video game training studies in older adults.

Disclosures: H. Li: None. **P. Wang:** None.

Poster

208. Brain Wellness and Aging: Pharmacological and Non-Pharmacological Interventions

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 208.07/D5

Topic: C.01. Brain Wellness and Aging

Support: Swedish Research Council (Medicine and Health, Grant 2018–03320)

Swedish Research Council (Natural and Engineering Science Grant 2018–05501)
The Swedish Brain Foundation
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Science for Life Laboratory (SciLifeLab)
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Title: Molecular mass spectrometry imaging reveals age-related changes of multiple neurotransmitter systems in response to acetylcholine esterase inhibition

Authors: E. FRIDJONSDOTTIR[1], T. VALLIANATOU[1], M. SHARIATGORJI[1], A. NILSSON[1], P. SVENNINGSSON[2], *P. E. ANDREN[1];
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Abstract: Aging is related to numerous neurochemical alterations and constitutes the preeminent risk factor for cognitive decline and neurodegenerative disorders, such as Alzheimer's disease (AD). The cholinergic system has been considered as the primarily affected neuronal system in AD. Acetylcholine esterase inhibitors, such as tacrine, i.e., therapeutic agents exerting their action by increasing the synaptic concentration of acetylcholine, are still the main treatment against AD. The cholinergic system has neuromodulatory effects and interacts with other neurotransmitters such as the dopaminergic and noradrenergic systems. The biochemical changes that occur with aging may have an altered effect on the response to acetylcholine esterase inhibition. We have recently developed a matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI) approach to investigate the distribution of neurotransmitters and their metabolites, e.g., the complete catecholamine system. That is, the method allows for quantitative mapping of multiple neurotransmitters simultaneously in a single tissue section. With a combination of the MALDI-MSI method and multivariate data analysis, we investigated the effect of normal aging and tacrine treatment on the cholinergic, dopaminergic, noradrenergic, serotonergic, histaminergic and GABAergic systems in multiple brain regions. Mice at age of 12 weeks (12-w) and 14 months (14-m) were studied. The animals received no treatment or a single dose of tacrine (10 mg/kg i.p.) (n=4 for all 4 groups). Coronal brain tissue sections were collected at brain levels containing major output structures of the targeted neurotransmitters systems. We found age-related neurochemical alterations in response to tacrine, especially regarding dopamine metabolism, acetylcholine and histamine. Tacrine treatment decreased 3-MT, a marker for dopamine release, in the striatum in the 14-m aged animals. Tacrine treatment also decreased the levels of norepinephrine in the hypothalamus, while it increased its metabolites, DOPEG and MOPEG, in the hypothalamus and in the cortex. The effects of tacrine treatment on changes in neurotransmitters were greater in the younger animals than in the old animals. In conclusion, the results highlight the multiplex effect mediated by acetylcholine esterase inhibition and the reduced response to treatment as a consequence of normal aging.

Disclosures: E. Fridjonsdottir: None. **T. Vallianatou:** None. **M. Shariatgorji:** None. **A. Nilsson:** None. **P. Svenningsson:** None. **P.E. Andren:** None.

Poster

208. Brain Wellness and Aging: Pharmacological and Non-Pharmacological Interventions

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 208.08/D6

Topic: C.01. Brain Wellness and Aging

Title: Interplay between 5-HT_4 receptors and GABAergic system within CA1 hippocampal synaptic plasticity

Authors: *M. BOULOUARD, P. LECOUFLET, J.-M. BILLARD, P. SCHUMANN-BARD, T. FRERET;
Univ. de Caen / INSERM, Caen, France

Abstract: The serotonin type 4 receptor (5-HT4R) has been described as a promising therapeutic target for learning and memory dysfunctions. Indeed, 5-HT4R activation promotes promnesic effects in adult rodents and leads to anti-amnesic effects either in aged animals or in pharmacologically-induced deficit condition as well as different transgenic models of Alzheimer disease. *In vivo* electrophysiological recordings within the hippocampus revealed a complex pattern of action of 5-HT4R on synaptic plasticity such as long term potentiation (LTP), depending on the concerned area. However, notably in the CA1 area, results obtained so far are divergent (e.g. both an increase or no effect on LTP of 5-HT4 agonists) and the mechanisms at work remain elusive. Using extracellular recordings in hippocampal slices from different young mice strains, we aimed to characterize the effects of RS67333 (5-HT4R agonist) and RS39604 (5-HT4R antagonist), on basal glutamatergic transmission and functional plasticity at CA3/CA1 synapses. 5-HT4R activation led to a strong decrease in paired-pulse facilitation (PPF) and LTP, while conversely promoted basal synaptic transmission and long term depression (LTD). The effects on long-term plasticity were antagonized by co-incubation with RS39604. This 5-HT4R antagonist did not have an effect alone whereas the effects on basal transmission persisted, suggesting that they are not mediated by the 5-HT4R. These results indicate that 5-HT4R exert a powerful modulation on functional plasticity including LTP and LTD in the CA1 area of mice hippocampus. Interestingly, preliminary data suggest that these effects are mediated through the modulation of inhibitory GABAergic tone.

Disclosures: **M. Boulouard:** None. **P. Lecouflet:** None. **J. Billard:** None. **P. Schumann-Bard:** None. **T. Freret:** None.

Poster

208. Brain Wellness and Aging: Pharmacological and Non-Pharmacological Interventions

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 208.09/D7

Topic: C.01. Brain Wellness and Aging

Support: Keck Foundation
NSF BRAIN Initiative
National Academies

Title: Diet modulates stability of brain networks in young adults

Authors: *L. R. MUJICA-PARODI[1], A. AMGALAN[2], S. F. SULTAN[3], S. SKIENA[3], A. LITHEN[1], N. ADRA[1], E. M. RATAI[4], N. A. SMITH[5], R. VEECH[6], K. CLARKE[7]; [1]Biomed. Engin., [2]Physics, Renaissance Sch. of Medicine, SUNY Stony Brook, Stony Brook, NY; [3]Computer Sci., SUNY Stony Brook, Stony Brook, NY; [4]A.A. Martinos Ctr. for Biomed. Imaging, Massachusetts Gen. Hosp., Charlestown, MA; [5]Ctr. for Neurosci. Res., Children's Natl. Hlth. Syst., Washington, DC; [6]NIAAA, NIH, Bethesda, MD; [7]Physiology, Anatomy, and Genet., Oxford Univ., Oxford, United Kingdom

Abstract: *Background*: Epidemiological studies suggest that insulin resistance accelerates progression of age-based cognitive impairment, which neuroimaging has linked to brain glucose hypometabolism. Ketones produce more ATP per unit oxygen than glucose. Here we test whether dietary changes are capable of modulating brain function, by switching neuronal Kreb cycle inputs from glucose to ketones, thereby increasing neuron-accessible ATP.
Methods: We first established *network stability* as a biomarker for brain aging using independent large-scale (N=292, 636, 18-80 years) 3T fMRI datasets. To determine if network stability was modulated by metabolic efficiency, we scanned 30 adults <50yrs using ultra-high-field (7T) ultra-fast (800ms) fMRI optimized for single-subject-level detection sensitivity. One sample was scanned under six dietary conditions: *fasting*, *dietary glycolytic* and *ketogenic* conditions ± *glucose bolus*. To isolate the impact of fuel source, an independent sample was scanned under fasting with calorically-matched *glucose* and *exogenous ketone ester* (β-hydroxybutyrate). We present the oldest subject (age 47) as a case study to illustrate detection sensitivity of neuroimaging-derived measures for early-stage insulin resistance at the single-subject level.
Findings: Across the lifespan, brain network destabilization correlated with decreased cerebral metabolic activity and cognitive acuity. The first effects became visible at around the age of 30 years, with the fastest degeneration seen at around the age of 60 years. Networks were destabilized by glucose and stabilized by ketones, irrespective of whether ketosis was achieved with a ketogenic diet or exogenous ketones. Our case study suggests that even as mild insulin resistance blunts network response to glucose, ketones retain their stabilizing effects.

Interpretation: Together, our results suggest that brain network destabilization reflects early signs of hypometabolism, a biomarker for brain aging. Dietary interventions that result in ketone utilization increase neurometabolic efficiency, and thus may show potential in protecting the aging brain.
Funding: WM Keck Foundation; NSF BRAIN Initiative; U.S. National Academies.

Disclosures: L.R. Mujica-Parodi: None. **A. Amgalan:** None. **S.F. Sultan:** None. **S. Skiena:** None. **A. Lithen:** None. **N. Adra:** None. **E.M. Ratai:** None. **N.A. Smith:** None. **R. Veech:** None. **K. Clarke:** None.

Poster

208. Brain Wellness and Aging: Pharmacological and Non-Pharmacological Interventions

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 208.10/D8

Topic: C.01. Brain Wellness and Aging

Support: Study Sponsor Applied Food Sciences Inc., Austin, Texas

Title: Guayusa induced neurotransmitter release in the rat prefrontal cortex

Authors: N. MORISOT[1], L. YU[1], A. MALIK[1], J. ROESER[1], G. PETERSEN[2], C. FIELDS[2], H. B. JANSSENS[1], ***A. RASSOULPOUR**[1];
[1]Charles River Labs., South San Francisco, CA; [2]Applied Food Sci., Austin, TX

Abstract: Sources of caffeine with beneficial antioxidant properties include teas that are derived from the holly *Ilex* species. These teas are unique and not related to green or black tea. Tea derived from *I. guayusa* is used by the Kichwa people in the Ecuadorian Amazon as a useful source of energy. Preliminary studies demonstrate that guayusa may impact neurotransmitters. The aim of this study was to examine the extracellular levels of the following neurotransmitters in the rat prefrontal cortex: dopamine (DA), norepinephrine (NE), serotonin (5-HT), GABA, glutamate (Glu), histamine (HA), acetylcholine (ACh) after administration of guayusa. This study demonstrated the increase of the neurotransmitters NE, 5-HT, HA, ACh and DA in the rat prefrontal cortex in a time-dependent manner. The extracellular level of GABA and Glu in the rat PFC was not significantly affected by the guayusa treatment. The consumption of guayusa as a tea may have a positive impact on cortical neurotransmitters.

Disclosures: N. Morisot: None. **L. Yu:** None. **A. Malik:** None. **J. Roeser:** None. **G. Petersen:** A. Employment/Salary (full or part-time):; Applied Food Sciences. **C. Fields:** A. Employment/Salary (full or part-time):; Applied Food Sciences. **H.B. Janssens:** None. **A. Rassoulpour:** None.

Poster

208. Brain Wellness and Aging: Pharmacological and Non-Pharmacological Interventions

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 208.11/D9

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant 1P30GM122733

Title: Aging, circadian rhythms, and cannabinoids

Authors: *E. L. HODGES, N. M. ASHPOLE;
Dept. of BioMolecular Sci., Univ. of Mississippi, University, MS

Abstract: Hormetic dose-response relationships suggest that opposing physiological responses can be driven by a single substance, however, detection and quantification of hormetic relationships is often difficult. One potential therapeutic application of hormesis is to the treatment of age-related circadian rhythm disorders. Studies in older humans and animals indicate that these daily physiological patterns are not consistent throughout the life span, and their disruption may influence pathologies of aging. Previous reports suggest that some cannabinoids, such as Δ9-Tetrahydrocannabinol (THC) and the endocannabinoid Anandamide may exhibit hormetic effects on body temperature and cognition in rodents. Additionally, these compounds regulate neuronal activity in the suprachiasmatic nuclei and systemic physiological signals known to entrain peripheral circadian rhythms. To determine whether the synthetic cannabinoid CP-55,940 exhibits hormesis and if this response changes with age, we examined the behavioral and physiological responses of 30 young (3m) and 30 old (22m) male C57BL/6 mice treated with varying doses of CP-55,940. All experiments were approved by UM-IACUC, and all testing was conducted in the dark under dim red light during the animals' active phase. To accurately quantify the effects of low-dose synthetic cannabinoid treatment, animals were thoroughly acclimated to handling, intraperitoneal injection of vehicle, and temperature recording prior to testing. Drug-induced changes in body temperature were measured using both rectal and infrared thermography, while locomotion and nociception were quantified via the Open Field and Hot Plate tasks, respectively. The preliminary results of these ongoing experiments indicate that locomotion, body temperature, and nociception are all significantly depressed following treatment with high doses of CP-55,940. When compared to vehicle treatment, lower doses of CP-55,940 trended to increase these measures from baseline. Future work will seek to establish a phase-response curve for this synthetic cannabinoid and investigate the chronobiotic potential of exogenous cannabinoids in aging mice.

Disclosures: E.L. Hodges: None. **N.M. Ashpole:** None.

Poster

208. Brain Wellness and Aging: Pharmacological and Non-Pharmacological Interventions

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 208.12/D10

Topic: C.01. Brain Wellness and Aging

Support: NIH R01 AG033288
NIH P50 AG05142

Title: Safety and feasibility of PhytoSERM - A selective β-receptor phytoestrogen formulation - for menopausal symptoms and cognitive decline: Phase 1b/2a clinical trial

Authors: *G. D. HERNANDEZ[1], Y. WANG[1], L. ZHAO[2], W. J. MACK[3], L. SCHNEIDER[3], R. D. BRINTON[1];
[1]CIBS, Univ. of Arizona, Tucson, AZ; [2]Sch. of Pharm., Univ. of Kansas, Lawrence, KS; [3]USC, Los Angeles, CA

Abstract: ***Background:*** Common symptoms during menopause are hot flashes, problems with memory and difficulty concentrating. Substantial biologic evidence supports the importance of estrogen for cognitive function. Given the fact that women account for 68% of all cases of Alzheimer's disease (AD), the role of estrogen and postmenopausal hormone replacement therapy (HRT) in cognitive impairment and AD is of considerable interest but evidence is mixed. Moreover, adverse outcomes associated with HRT have led to increasing number of women declining its use but seeking non-pharmaceutical alternatives, whichmight prove to be symptomatically effective and cognitively enhancing.PhytoSERM is a formulation composed of rationally-selected estrogen receptor-β (ERβ) phytoestrogens: genistein, daidzein, and S-equol. Its selective affinity for ERβ may enhance neuron function and estrogenic mechanisms in the brain without having peripheral estrogenic activity.We report here outcomes of a phase 1b/2a clinical trial that served several purposes in the development of the PhytoSERM formulation, including dose-ranging, assessment of tolerability and safety, pharmacokinetic profiling, and testing its feasibility and potential for efficacy over 12 weeks. ***Methods:*** We conducted a randomized, placebo-controlled trial of 12 weeks duration comparing 50 and 100 mg of PhytoSERM with placebo for non-cognitively impaired, perimenopausal women ages 45 to 60, with intact uteri and ovaries, at least one cognitive complaint, and one vasomotor-related symptom. Primary objectives were to assess safety and tolerability of both oral doses of PhytoSERM taken daily. Secondary objectives were to evaluate potential indicators of efficacy on cognition and vasomotor symptoms over 12 weeks. ***Results:*** Seventy-one women were randomized to treatment; 70 were evaluated at 4 weeks and 5 did not complete 12 weeks. Overall, 87% were greater than 90% compliant with their medication. There were no statistically significant effects on either vasomotor composite or the neuropsychological composite over 12

weeks (p=0.25 and p=0.57, respectively. However, change from baseline over 12-weeks in hot flash frequency alone was significantly lower in the 50mg group compared to placebo (p=0.04). Adverse events occurred in 16.7% (n=4) placebo, 39.1% (n=9) 50 mg per day, and 29.2% (n=7) 100 mg per day treated participants. ***Conclusion:*** The PhytoSERM formulation appeared safe and well-tolerated at 50 and 100 mg daily doses. Based on safety outcomes, and vasomotor symptoms and cognitive outcomes at 12 weeks an optimal daily dose of 50mg was established for a phase 2 efficacy trial.

Disclosures: G.D. Hernandez: None. **Y. Wang:** None. **L. Zhao:** None. **W.J. Mack:** None. **L. Schneider:** None. **R.D. Brinton:** None.

Poster

208. Brain Wellness and Aging: Pharmacological and Non-Pharmacological Interventions

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 208.13/D11

Topic: C.01. Brain Wellness and Aging

Support: USDA Intramural

Title: Individual and synergistic effects of walnut oil and blueberry treatments on reducing neuroinflammation in rat microglial cells *in vitro*

Authors: D. R. FISHER, D. S. CAHOON, ***B. SHUKITT-HALE**;
USDA-ARS Human Nutr. Res. Ctr. on Aging, Boston, MA

Abstract: With age, increased generation of free radicals and decreased endogenous defense enzymes result in oxidative stress and pro-inflammatory signals that ultimately disrupt or destroy cells. Age-related neurodegeneration and behavioral declines have been associated with increases in neuroinflammation and oxidative stress in the brain. Therefore, plant polyphenols high in antioxidant and anti-inflammatory activity, such as those in berries and nuts, may prevent or reduce the neurochemical and behavioral declines that occur in aging. Previous *in vitro* and animal studies from our laboratory have shown that blueberries and walnuts are able to attenuate markers of neuroinflammation as well as age-related behavioral deficits. While blueberries and walnuts each provide neuroprotective dietary components, they also may act synergistically to enhance the effects seen with individual supplementation due to their unique compounds. Therefore, relative to individual treatments, combined walnut oil/blueberry (WO/BB) treatments may: 1) have increased beneficial effects due to synergistic mechanisms; 2) have the same effects due to overlapping additive properties; or 3) have a reduced beneficial effect, as the individual doses are lower, or due to another unknown mechanism. This study investigated the *in vitro* protective effects of blueberry, walnut oil, and combined blueberry/walnut oil treatments on LPS-induced neuroinflammation in rat microglial cells at various concentrations and treatment

durations. HAPI rat microglial cells were treated with freeze-dried blueberry extract, walnut oil, or WO/BB at 0.05, 0.1, 0.2, 0.5 and 1.0 mg/mL for 48 hours, 1 week, 2 weeks and 4 weeks. At the end of each treatment time point, cells were stressed with LPS at 100 ng/mL overnight and expression of nitric oxide, TNFα, COX-2 and iNOS were measured as inflammatory indices. Results showed that blueberry and walnut oil treatments reduced LPS-induced neuroinflammation in a concentration- and time-dependent manner. However, treatment with blueberry had a stronger effect on reducing neuroinflammation than WO/BB, and both blueberry and WO/BB were more effective than walnut oil. This suggests that the blueberry in the combined treatment is primarily responsible for the beneficial effects of WO/BB, and blueberry and walnut oil do not act synergistically to reduce neuroinflammation.

Disclosures: D.R. Fisher: None. **D.S. Cahoon:** None. **B. Shukitt-Hale:** None.

Poster

208. Brain Wellness and Aging: Pharmacological and Non-Pharmacological Interventions

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 208.14/D12

Topic: C.01. Brain Wellness and Aging

Support: USDA/ARS intramural grant
The Robert and Margaret Patricelli Family Foundation

Title: Blueberry extract attenuates the *in vitro* expression of oxidative stress and inflammation markers in adult human neural progenitor cells

Authors: D. F. BIELINSKI, B. SHUKITT-HALE, D. STEINDLER, ***T. ZHENG**;
Neurosci. & Aging Lab., USDA Human Nutr. Res. Ctr, Tufts Univ., Boston, MA

Abstract: The aging process impacts neural stem cells and causes a significant decline in neurogenesis that contributes to neuronal dysfunction. Previous studies suggest that oxidative stress and inflammation are major factors leading to the deleterious effects of aging and the development of age-related neurodegenerative diseases, and that naturally occurring phytonutrients and bioactives appear to decrease oxidative stress and inflammation, and possess both neuroprotective and neurogenic properties. Blueberries are rich in polyphenols and have been shown to improve cognition and memory in both aged animals and humans. Our previous data indicate that blueberry supplementation can increase neurogenesis in aged rodents, and has beneficial effects on the viability and proliferation of adult human neural progenitor cells (AHNPs). The current study investigates the effects of blueberry treatments on the expression of oxidative stress and inflammation markers in AHNPs, in order to further establish precise cellular and molecular mechanisms underlying the nutrient beneficial effects of such foods on human neural stem and progenitor cells. AHNPs isolated from human hippocampus were

cultured in N2 medium plus 5% fetal bovine serum, bovine pituitary extract, and growth factors. To determine whether blueberries could protect AHNPs from oxidative stress and inflammation induced by a cellular stressor, dopamine (DA), AHNPs were treated with freeze-dried blueberry extract at concentrations from 0.1 to 0.5 mg/ml for 7 days followed by exposure to DA (0.1 μm) for 2 hours. The expression of oxidative stress and inflammation markers such as iNOS and Nox-2 was then evaluated using western blot analysis of cell lysates. A subset of treated cells was also immunofluorescently labeled with antibodies against these markers to confirm their expression before and after DA-induced stress. Our data indicate that DA-induced stress increased the expression level of both iNOS and Nox-2 in hippocampal AHNPs, and that the blueberry treatments were able to attenuate the iNOS expression level by 10-25%, and the Nox-2 expression level by 15-50%, compared to the non-treated cells. Therefore, polyphenol-rich berry extracts may confer anti-oxidative and anti-inflammatory benefits on AHNPs, suggesting a potential neuroprotective role for particular dietary nutrients in helping to prevent neural cell loss during aging and thus slow progression of neurodegenerative diseases including Alzheimer's and Parkinson's.

Disclosures: **D.F. Bielinski:** None. **B. Shukitt-Hale:** None. **D. Steindler:** None. **T. Zheng:** None.

Poster

208. Brain Wellness and Aging: Pharmacological and Non-Pharmacological Interventions

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 208.15/D13

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: DA32444
DA15014
DA040519

Title: A novel strategy to precisely target individual steps in the APP amyloidogenic processing

Authors: ***R. BRANDIMARTI**[1,2], J. LUCHETTA[1], I. G. ERALP[1], O. MEUCCI[1];
[1]Pharmacol. and Physiol., Drexel Univ. Col. of Med., Philadelphia, PA; [2]Pharm. & Biotech., Univ. of Bologna, Bologna, Italy

Abstract: Altered Amyloid Precursor Protein (APP) processing and β-amyloid (Aβ) production have long been recognized as major factors in the cognitive decline associated with neurological disorders such as Alzheimer's disease (AD) and HIV-Associated Neurocognitive Disorders (HAND). However, recent studies show that a linear link between Aβ accumulation and disease progression - as proposed in the original "amyloid cascade hypothesis"- is an over-simplified explanation for these complex disorders. Events leading to APP maturation and processing rely

on sophisticated molecular machinery that regulates its trafficking, post-translational modification, and cleavage. Therefore, molecular tools able to precisely affect these processes represent a powerful approach to deepen our understanding of the actual contribution of individual events to neuronal demise, and possibly relieve the burden of these devastating disorders. Building on our previous findings of the molecular properties of US9 (a Herpes Simplex Virus transport protein), and of its overlapping trafficking behavior with APP, we designed several US9-based molecular tools able to manipulate APP processing by either direct or indirect modalities. These chimeric proteins exploit the driving capabilities of US9 to locate functional cargos next to APP in specific cellular compartments. Here we describe the effects of two US9 chimeras with enzymatic or non-enzymatic activity. In the first construct, the peptidase domain from Adam10 was fused to the C-terminal portion of US9 in order to locate its α-secretase activity in the lumen of endosomal compartments. In the second construct, the binding domain from the APP interacting protein X11 was attached to the US9 N-terminus to drive its expression on the cytosolic side of the vesicle and in close proximity to the APP C-terminal domain. When expressed in cultured human cells and primary rat neurons, both US9-targeted functionalities reduced APP β-cleavage and amyloidogenic load. Additionally, soluble αAPP fragments with neuroprotective and neurotrophic properties were released from transduced cells. These results demonstrate that the successful reduction of APP β-cleavage and Aβ production can be effectively achieved with US9-based constructs that rely on different mechanisms, as either enzymatic or non-enzymatic activity is present in the two chimeric proteins described here. Furthermore, our studies prove the ability of these molecular tools to precisely manipulate APP processing in multiple ways at individual steps.

Disclosures: **R. Brandimarti:** None. **J. Luchetta:** None. **I.G. Eralp:** None. **O. Meucci:** None.

Poster

209. Brain Wellness and Aging: Systemic Factors and Brain Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 209.01/D14

Topic: C.01. Brain Wellness and Aging

Support: Groff Foundation
Collin and Lilli Roche 1993 Student Research Program

Title: Short-term chronic systemic inflammation affects spatial memory and central inflammation throughout adulthood

Authors: M. B. FREY[1], *L. L. WILLIAMSON[2];
[1]Williams Col., Williamstown, MA; [2]Biol. Sci., Northern Kentucky Univ., Highland Heights, KY

Abstract: Neuroinflammation, aging, and memory decline are highly correlated. Aging, along with inflammatory disease and head trauma, often causes microglial cells to become “primed”, or overly sensitive to insult. Priming can cause microglia to overreact to little or no insult and to sustain inflammatory activities longer than necessary, damaging nearby healthy tissue and interrupting proper memory function. To investigate the interactions between neuroinflammation, sex, aging, and memory in Sprague-Dawley rats, we induced chronic systemic inflammation at 3 age points (3 -, 6 - and 12-months-old) in males and females. We tested contextual memory and molecular markers of inflammation. Rats were given 5 once-daily injections: lipopolysaccharide (LPS; 250-350ug/kg) to induce chronic inflammation, or saline control, and trained and tested in the Context-Object Discrimination task before tissue collection. We found differential effects of short-term, chronic systemic inflammation across 3-month-old (*n*=34), 6-month-old (*n*=40), and 12-month-old rats (*n*=33) in contextual learning and memory. There was a significant interaction of age and treatment, such that saline-treated groups showed a significant decline in learning across aging. The saline-treated 12-month-old animals performed significantly worse than both 6-month-olds and 3-month-olds and the same as LPS-treated 12-month-olds. Surprisingly, 6-month-olds demonstrated improved contextual memory in response to systemic inflammation, while LPS-treated 3-month-olds performed significantly worse than saline-treated controls. Along with these significantly different cognitive responses to peripheral inflammatory insults, there were significant differences in hippocampal IL-1beta protein expression and phosphorylated tau concentrations across age group and sex, indicating variable levels of neuroinflammation, although peripheral IL-1beta levels did not differ across age 5 days after inflammation. Age clearly has a significant impact on the brain’s response to similar peripheral inflammatory insults and is an important factor that must be considered when developing animal models.

Disclosures: M.B. Frey: None. **L.L. Williamson:** None.

Poster

209. Brain Wellness and Aging: Systemic Factors and Brain Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 209.02/D15

Topic: C.01. Brain Wellness and Aging

Support: JSPS KAKENHI Grant Number JP18K05342

Title: Humanin, a bioactive peptide, increases hippocampal acetylcholine level in normal mice

Authors: N. IKEGAWA, M. MURAKAMI, *T. NIIKURA;
Sophia Univ., Tokyo, Japan

Abstract: Humanin(HN) is a 24-residue endogenous peptide. HN was identified as a neuroprotective factor that suppresses cell death associated with Alzheimer's disease such as amyloid beta. HN is secreted from cells, and systemically distributes via blood stream. Circulating level of HN decreases age-dependently in human and rodents, implicating the relationship of HN to aging-associated physiological changes. S14G-HN, HN in which 14th serine is replaced by glycine, is a highly potent HN derivative. S14G-HN has 1000-fold higher potency than HN and suppressed cognitive impairment in Alzheimer's disease model mice. S14G-HN also ameliorates memory impairment caused by muscarinic receptor antagonists in normal mice. However, the functional mechanism underlying the effect of HN on cognitive activities is still unclear.To understand the mechanism of HN-induced improvement in cognitive function, we focused on the levels of neurotransmitters in the brain. We examined the acetylcholine level in the normal mouse brain under the free moving condition. We placed microdialysis probe near the hippocampal region and collected samples through interstitial liquid for 210 min after intraperitoneal injection of S14G-HN or vehicle. The amount of acetylcholine in the sample was measured using high performance liquid chromatography. Vehicle injection did not cause significant change in the acetylcholine level throughout the period. On the other hand, the level of acetylcholine was increased by 1.5-fold after 30 min of S14G-HN administration compared with pre-administration level and the level was maintained for 180 min. The physical activity assessed by the walking distance was similar in two groups, indicating that the effect of S14G-HN on the acetylcholine level was unaffected by the locomotor activity. We also measured catecholamine levels and found an increasing trend of catecholamine levels in the hippocampal region by S14G-HN injection. This finding suggests that the effect of S14G-HN is not specific to acetylcholine. From these results, it is assumed that HN can modulate neurotransmitter release in the normal mouse brain.

Disclosures: **N. Ikegawa:** None. **M. Murakami:** None. **T. Niikura:** None.

Poster

209. Brain Wellness and Aging: Systemic Factors and Brain Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 209.03/DP05/D16

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: C.01. Brain Wellness and Aging

Title: Sleep-wake cycle, cognitive functions and adult neurogenesis during aging in a non-human primate

Authors: *J. ROYO, O. ZOLARIO, F. AUJARD, F. PIFFERI;
UMR 7179 Adaptative Mechanisms and Evolution, Natl. Museum of Natural History (MNHN)/French Ctr. for Scientific Res. (CNRS), Brunoy, France

Abstract: The process of neurogenesis has been demonstrated to persist throughout life in restricted brain areas such as the dentate gyrus of the hippocampus and the subventricular zone of the lateral ventricles.
The newly born neurons may be involved in memory processes and mitigate the effects of cognitive decline during aging. This adult plasticity can be disturbed by endogenous and exogenous factors.
Among these factors, sleep seems to play an important role in brain physiologic processes such as synaptic plasticity and memory functions. Several studies have shown that the alteration of sleep-wake cycle may have cumulative effects leading to a major decrease in neurogenesis and mnesic impairment. In the present study, we are interested in studying the relationship between sleep, memory function and neurogenesis process by comparing sleep-wake cycles in an adult non-human primate, the grey mouse lemur, during aging. Twenty young adults (1 - 5.5 years) and thirteen aged adults (> 5 years) were evaluated on visual discrimination task designed to measure learning and memory abilities. Some animals were used to measure the impact of aging on sleep-wake cycles (12 young and 4 aged animals) and neurogenesis process (7 young and 8 aged mouse lemurs). During aging, there were large interindividual variabilities among animals showing the existence of two populations with opposite success rates. Some aged animals presented a specific deficit in learning and memory functions whereas other aged mouse lemurs had cognitive performances equivalent or better than younger animals. The analysis of the alterations of sleep-wake cycle and the process of neurogenesis during aging is still in progress. By knitting together these different parameters, our data will help us to understand if the effects of sleep on cognitive functions may possibly be mediated by a change in adult neurogenesis. Newly born neurons produced during sleep would prevent the alteration of cognitive functions with age. The understanding of the mechanisms of aging will be useful for setting up new treatments for cognitive impairments and brain disorders during aging.

Disclosures: **J. Royo:** None. **O. Zolario:** None. **F. Aujard:** None. **F. Pifferi:** None.

Poster

209. Brain Wellness and Aging: Systemic Factors and Brain Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 209.04/D17

Topic: C.01. Brain Wellness and Aging

Support: R01 AA025380

Title: Sex comparisons of binge ethanol-induced neurodegeneration in a rodent model

Authors: *R. K. WEST, J. L. LEASURE;
Psychology, Univ. of Houston, Houston, TX

Abstract: In the U.S., 1/6 of adults report binge drinking about 4 times a month. There is also mounting evidence indicating that females may be more vulnerable to the neurotoxic effects of ethanol than males. We have recently shown that weekly binge ethanol (5 g/kg) administration for 11 weeks increases partial activation (priming) of microglia in the hippocampus and causes significant dentate gyrus (DG) cell loss despite an increase in neurogenesis in female rats. However, it is not known whether damage occurs with fewer weeks of exposure, nor whether the emergence or magnitude of cell loss is sex-dependent. We hypothesized that cellular damage would be greater and detectable earlier in female rats. Adult Long-Evans rats (80 male and female) were administered 5g/kg ethanol (or an iso-caloric control dose) via intra-gastric gavage once-weekly for 3 or 8 weeks. Neither BEC (174 mg/dl) nor behavioral intoxication measures differed over time, indicating that tolerance did not occur. Male rats, however, acted more behaviorally intoxicated than females. Brains were collected 4 days following the final ethanol dose, and immunohistochemically processed for neurons (NeuN) and microglia (Iba1). Stereology was used to quantify target cell populations in the hippocampus and medial prefrontal cortex (mPFC). After 3 weeks, binge ethanol significantly decreased the number of NeuN+ cells in the DG of female rats in comparison to controls. After 8 weeks, both male and female binged rats had significant DG cell loss. Both 3 and 8 weeks of binge ethanol significantly increased the total number of microglia (Iba1) in the hippocampus in males and females. After 8 weeks, the number of primed microglia in the hippocampus increased in both sexes. In the mPFC, 8 weeks of binge ethanol increased microglial priming and total microglial number in both sexes. Our results show hippocampal cell loss and an increased inflammatory response in ethanol-vulnerable regions following repeated binge exposures and demonstrate that females manifest significant hippocampal cell loss earlier than males from a weekly binge model. Ongoing work investigates sex differences in behavioral outcomes due to repeated binge exposures. (R01 AA025380)

Disclosures: R.K. West: None. **J.L. Leasure:** None.

Poster

209. Brain Wellness and Aging: Systemic Factors and Brain Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 209.05/D18

Topic: C.01. Brain Wellness and Aging

Support: Health and Medical Research Fund – Advanced Medical Research 04151216.

Title: Age-dependent changes in the periodontium and the neuropathological features of 3xTg mice as well as the impact of periodontitis on Alzheimer's disease

Authors: *R. P. H. WANG[1], S. S. Y. ZHANG[1], X. X. WANG[2], W. K. LEUNG[2], J. Y. S. HO[4], R. C. C. CHANG[1,3];
[1]Lab. of Neurodegenerative Diseases, Sch. of Biomed. Sciences, LKS Fac. of Med., [2]Fac. of Dent., [3]State Key Lab. of Brain and Cognitive Sci., The Univ. of Hong Kong, Hong Kong, China; [4]Sch. of Nursing, Fac. of Hlth. and Social Sci., The Hong Kong Polytechnic Univ., Hong Kong, China

Abstract: Alzheimer's disease (AD) is the most common form of dementia characterized by the presence of amyloid plaque and neurofibrillary tangles. Over the years, neuroinflammation has emerged to be the third core pathology of AD that plays a central role in the disease pathogenesis. This response is characterized by activation of microglia and astrocytes. Emerging epidemiological studies have shown that peripheral immune activation can lead to neuroimmune responses through activation of glial cells. One of the common sources of chronic systemic inflammation is periodontitis, which is an advanced form of periodontal disease induced by oral pathogenic bacteria, leading to alveolar bone loss and ultimately tooth loss. As periodontitis is the major oral health problem in both elderly and AD patients, it suggests that it may intensify the neuroimmune responses in AD and exacerbate the disease conditions. While aging is the major risk factor for AD, other studies also confirmed that occurrence and severity of periodontal destruction increases with age. One of the reasons is that advancing age leads to dysregulation of the immune system. By employing Micro-CT (micro-computed tomography) scan, our data revealed that in comparison to young 3xTg mice, old mice displayed significantly ($p < 0.05$) increased periodontal bone loss. In addition, as the severity of AD pathology increases with age in 3xTg mice, cognitive functions of these mice were also examined. Our results showed that aged mice displayed a worse cognitive performance. Increased neuropathology in the brains of aged 3xTg was also observed. Coincidently, the increase in periodontal bone loss and worsened cognitive functions in aged 3xTg AD mice led us to question whether periodontitis would induce and/or exacerbate neuroimmune responses and cognitive impairments. To address the question, female mice at 6 months old were injected with heat-killed *P. gingivalis* bacteria into their buccal mucosa three times per week every other week for a total of 6 weeks to establish periodontitis. After infection, cognitive functions of mice were assessed and some had undergone MicroPET scan, which is a unique technology that measures the brain glucose metabolism. Also, significant increase of the pro-inflammatory cytokines IL-1β and IL-6 were found in the frontal cortex, MCP-1 in the hypothalamus and IL-1β in the hippocampus of mice after periodontal infection. Together, these findings prove the relevance of the current periodontitis model to further examine neuroimmune responses and AD pathologies in the brains of 3xTg mice.

Disclosures: R.P.H. Wang: None. **S.S.Y. Zhang:** None. **X.X. Wang:** None. **W.K. Leung:** None. **J.Y.S. Ho:** None. **R.C.C. Chang:** None.

Poster

209. Brain Wellness and Aging: Systemic Factors and Brain Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 209.06/D19

Topic: C.01. Brain Wellness and Aging

Support: CVAMC VISN 10 Research Initiative Program

Title: Targeted metabolomics of mouse brain - Small phenolic molecules from gut microbiota

Authors: M. E. OBRENOVICH[1], C. J. DONSKEY[2], ***G. E. JASKIW**[3];
[1]Res. Service, [2]Infectious Dis. Service, [3]Louis Stokes Cleveland DVAMC, Cleveland, OH

Abstract: Dysregulated urinary levels of numerous small phenolic molecules (SPMs) such as 3-(3-Hydroxyphenyl)-3-hydroxypropionic acid (3,3-HPHPA), 3,3-hydroxyphenlypropionic acid (3,3-HPPA), 3-hydroxybenzoic acid (3-HBA), 3-hydroxyhipppuric acid (3-HHA) and others have been associated with neuropsychiatric disorders including schizophrenia or autism spectrum disorder (Jaskiw et al, 2019). Many of these SPMs cannot be generated by endogenous mammalian cells but can be synthesized by gut microbiota (GMB). Applying a high-performance liquid chromatography tandem mass spectrometry (LC/MS) metabolomics approach, we recently demonstrated that human cerebrospinal fluid contains numerous SPMs, including those produced by the GMB (Obrenovich et al, 2018). Thus, brain levels and brain effects of these SPMs merit characterization. Since such studies typically involve rodent models, we applied the LC-MS metabolomics approach to mouse brain. Female CF-1 mice (25 - 30 g) received injections (400 mg/kg IP) of L-tyrosine (L-TYR) or L-phenylalanine (L-PHE), both known precursors of many SPMs. Brains were harvested 2 - 8 h later. Technical parameters were similar to those previously described (Obrenovich et al, 2018). Preliminary data analysis indicates that mouse brain contains numerous SPMs (e.g. 4-hydroxyphenyllactic acid, 3-hydroxyphenylpyruvic acid (3-HPPU), 4-HPPU, isomers of HPPA, 3,3-HPHPA, isomers of dihydroxyhydrocinnamic acid, hippuric acid, 3-HBA) as well as other GMB-associated small molecules (cinnamoylglycine, indoxyl sulfate, taurine, trimethylamine N-oxide) and dietary polyphenols (catechin, hesperidin). Systemic administration of L-TYR or L-PHE elevated brain levels of a number of SPMs. Thus, many of the same GMB-derived chemicals identified in the human central nervous system, are also present in mouse brain. This justifies the use of the mouse model to characterize the kinetics and neuroactivity of SPMs and other GMB-derived molecules. The process may yield biomarkers of value in conditions such as schizophrenia or autism spectrum disorder.

Disclosures: M.E. Obrenovich: None. **C.J. Donskey:** None. **G.E. Jaskiw:** None.

Poster

209. Brain Wellness and Aging: Systemic Factors and Brain Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 209.07/D20

Topic: C.01. Brain Wellness and Aging

Support: United Kingdom Biotechnology and Biological Sciences Research Council grant BB/N009088/1
United Kingdom Medical Research Council grant MR/N013700/1

Title: Multimodal MR imaging of environmentally enriched and diet restricted rat model of healthy ageing

Authors: *E. MACNICOL, K. RANDALL, C. SIMMONS, E. KIM, M. BERNANOS, F. E. TURKHEIMER, D. CASH;
Neuroimaging, King's Col. London, London, United Kingdom

Abstract: Age-related cognitive decline varies between individuals and is believed to be influenced by various factors, including lifestyle (e.g. intellectual engagement, fitness, and caloric restriction). A rat model of 'healthy ageing' was implemented by combining environmental enrichment and dietary restriction (EEDR). Rats underwent magnetic resonance imaging (MRI) at four points across their lifespan, with the aim of exploring longitudinal changes that best correlate with healthy ageing.
Male Sprague-Dawley rats (n=48) were scanned with a 9.4 T Bruker BioSpec MRI scanner at 3-, 5-, 12-, and 18- months of age with a comprehensive protocol. The multi-modal metrics collected included diffusion and T_1-weighted structural images, relaxometry (T_1, T_2, and T_2*) maps, and blood oxygen level dependent (BOLD) and continuous arterial spin labelling (CASL) functional images. EEDR was implemented for half of the cohort after the first scan, by providing a rotation of cage toys changed weekly and imposing 24 hours of fasting every other day.
Subtle structural differences emerged between the groups after the implementation of EEDR. Total intracranial volume was larger in control rats, with a significant difference at the first time point after the intervention ($p=0.0354$, $d=-0.756$). This observation is likely driven by an increased volume of grey matter in controls, which was significantly different at the time points following the intervention, at 5- and 12-months of age ($p=0.0308$, $d=-0.77$ and $p=0.0426$, $d=-0.124$ respectively). However, when exploring estimated grey matter volume in a voxelwise analysis, there are various regions not explained by controlling for brain size.
Further analysis will explore how the multi-modal MRI dataset can best provide information regarding differences seen in brain structure between the groups. Consequently, the implications for brain function in the context of healthy ageing will be explored.

Disclosures: **E. MacNicol:** None. **K. Randall:** None. **C. Simmons:** None. **E. Kim:** None. **M. Bernanos:** None. **D. Cash:** None. **F.E. Turkheimer:** None.

Poster

209. Brain Wellness and Aging: Systemic Factors and Brain Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 209.08/D21

Topic: C.01. Brain Wellness and Aging

Support: NASA grant #NNX15AJ20H
Herbert H. and Grace A. Dow Scholars Award

Title: Understanding the impact of chronic low-dose, low energy, proton radiation on systemic inflammation and anxiety-like behaviors in mice

Authors: *P. NOLTE[1], V. PARKER[1], P. A. DEYOUNG[2], P. D. RIVERA[1];
[1]Dept. of Biol., [2]Dept. of Physics, Hope Col., Holland, MI

Abstract: A major component of NASA's 2018 strategic plan was to send astronauts to and beyond our lunar orbit within the next couple of decades. A risk to mission success is an astronauts' exposure to galactic cosmic radiation (GCR), a mixture of chronic low-dose, high-energy, high-charge ion particles (HZE). Previous high-energy radiation proton studies show lasting inflammation in the eye in humans treated for uveal melanomas. In mice, HZE particles also showed deficits in cardiac physiology, brain electrophysiology, and memory. Of particular interest to long-term mission success are low-dose, low-energy protons due to their high abundance in the space environment. Given the detrimental physiological and cognitive impact on humans and rodents after high-energy proton studies and a lack of low-energy proton studies on skin and inflammation, knowledge of how inflammation might respond to chronic low-dose, low-energy proton radiation is warranted. In our experiment, mice were put into a 50mL conical tube; half were irradiated using the Hope College Pelletron accelerator at a low-dose (~2 mGy/wk) of protons. After 10 weeks, half the irradiated mice and half the non-irradiated mice were euthanized for molecular studies. Levels of inflammatory cytokines like tumor necrosis factor, which are associated with increased depression, bipolar disorder and schizophrenia were assessed. The other half underwent behavioral tests that looked at stress behaviors. Therefore, the proposed study aimed to test the hypothesis that chronic low-dose, low-energy proton radiation negatively impacts mental health due to lasting systemic inflammation. Future directions are to examine HZE particles (e.g. Fe, Si, and C) to compare chronic low-energy low-dose particles and high-energy low-dose protons which will help future NASA missions to and beyond lunar orbit.

Disclosures: **P. Nolte:** None. **V. Parker:** None. **P.A. DeYoung:** None. **P.D. Rivera:** None.

Poster

209. Brain Wellness and Aging: Systemic Factors and Brain Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 209.09/D22

Topic: C.01. Brain Wellness and Aging

Support: Ramalingaswami fellowship (BT/RLF/Re-entry/07/2014)
Ramalingaswami fellowship (BT/RLF/Re-entry/31/2011)
NBRC core funds

Title: Alpha peak frequency shifts: The long and short of it

Authors: *A. PATHAK, D. ROY, A. BANERJEE;
Cognitive Brain Dynamics Lab., Natl. Brain Res. Ctr., Gurgaon, India

Abstract: The human brain rhythm has been observed to undergo shifts in its peak alpha frequency(PAF) in response to task conditions. While task dependent shifts in PAF occur at fast time scales, slow shifts in spontaneous PAF with ageing have also been reported. We hypothesize that the age related changes in PAF arise from alterations in the structure of thalamocortical connectivity. On the other hand, spontaneous switches in PAF are suggestive of multistable dynamics. Here we seek to explain both the slow(longitudinal) and fast(task dependent) shifts in PAF as arising from a unified model of thalamocortical dynamics. According to our hypothesis, age related changes in PAF are caused by changes in myelination strength in the connections between the thalamic nuclei and cortex, while task dependent switches in PAF occur due to noise driven excursions between multistable modes of the thalamocortical system. Since the computational model of the thalamocortical system admits of multiple parameters and therefore, multiple ways of modulating PAF, we imposed the additional constraint that the amplitude of alpha band must remain invariant to quickening/slowing of PAF. This empirically driven constraint has the effect of narrowing the search space for valid mechanisms.
For our purposes we invoke a well studied thalamocortical model, first developed by Robinson et. al. to study spontaneous alpha activity. The model consists of three neural populations out of which 2 populations are cortical and the other 2 correspond to thalamic nuclei(specific and reticular). In brief, the model studies the interaction of cortical excitatory and inhibitory neurons with the relay and reticular neurons present in the thalamus where the mean field responses of each population are the variables of interest. The model consists of a set of first order nonlinear differential equations that include synaptic and dendritic dynamics, nonlinear firing responses and axonal delays. The model exhibits hopf bifurcations on appropriate choice of parameters. The dynamical repertoire of the system can include multistable solutions each of which

correspond to different output frequencies. Such frequency states can be conceptualized as distinct modes which are a function of noise parameters or external inputs.

Disclosures: **A. Pathak:** None. **D. Roy:** None. **A. Banerjee:** None.

Poster

209. Brain Wellness and Aging: Systemic Factors and Brain Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 209.10/D23

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant 1R15AG051940-01A1

Title: Case study of an aging monkey pre and post mortem: Cognitive decline, Alzheimer's disease markers, immunological response, and neurogenesis

Authors: *J. J. NEIWORTH[1], C. LEPPINK-SHANDS[2], C. LIPHART[1];
[1]Psychology/Neuroscience, [2]Psychology, Carleton Col., Northfield, MN

Abstract: The study provides a case analysis of a 20-year old cotton top tamarin monkey (*Saguinus oedipus*), a species found to acquire beta amyloid plaques and hyperphosphorylated tau by age 12.5 in an age-dependent manner (Lemere et al., 2008). Our lab has confirmed independently an age-dependent increase in amyloid plaque accumulation, taupathology, and reactive astrocytes in 4 monkeys ranging in age from 8-24 post mortem in areas related to neurodegenerative disease, including entorhinal cortex, hippocampus, and subiculum. Our current study involves testing each live monkey (n=9) on working memory, visuospatial search and long term memory rule switching tasks repeatedly as they near the end of their lives, and then examining levels of Alzheimer's disease markers, immune response, cell degeneration, and neurogenesis post mortem. In the case presented here, the individual's data from tests of category switching and visual search are compared to younger monkeys and older monkeys, and we correlate cognitive decline with physiological markers through immunohistochemical techniques and stereology post mortem. The goal is to build a complete record of age-related cognitive skills and loss and physiological changes in tamarins to determine if neurodegeneration is common and produces similar cognitive and physiological issues as those found in humans. In this individual, there were difficulties in switching rules once one was learned, and in finding visual targets in conjunctive displays which had distractors with overlapping features. Through stereology examination post mortem, we found beta amyloid plaques, some tau pathology, reactive astrocytes, dystrophic microglia, and evidence of neurogenesis in some areas of the brain, not exclusive to the dentate gyrus.

Disclosures: **J.J. Neiworth:** None. **C. Leppink-Shands:** None. **C. Liphart:** None.

Poster

209. Brain Wellness and Aging: Systemic Factors and Brain Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 209.11/D24

Topic: C.01. Brain Wellness and Aging

Support: Psi Chi International Graduate Research Grant

Title: The effects of antibiotics and probiotics on memory, depression, and the hippocampus across age groups

Authors: *A. G. POWELL, R. M. FARMER, R. P. FORD, E. M. GOTT, C. M. GREGG, A. R. KNIFFIN, S. M. MOSELEY, V. A. WRIGHT, E. A. ZIHAL, M. L. SHOUP-KNOX; Psychology, James Madison Univ., Harrisonburg, VA

Abstract: The brain requires nutritional information from the gut in order to regulate various neurocognitive factors and the behavior of the organism (Collins & Bercik, 2009). Therefore, changes in the diversity of the gut microbiome have been linked to the development of depression and anxiety, exhibited in both animals (e.g., Gareau et al., 2011) and humans (e.g., Naseribafrouei et al., 2014). These studies have demonstrated the neural and behavioral effects of altering the gut microbiome via antibiotics to diminish gut flora diversity and probiotics to reestablish gut flora diversity. As people age, a decrease in gastrointestinal tract functioning occurs, also resulting in changes to the gut microbiome (Kato et al., 2017). More specifically, a decrease in diversity of commensal bacteria and an increase in the diversity of harmful bacteria are observed with ageing, and are associated with reduced cognitive performance (O'Toole & Jeffrey, 2015). Therefore, the usage of antibiotics contributes to the loss of gut bacteria diversity, and may augment the cognitive-related deficits observed in older adults. The current study examined the effects of antibiotic and probiotic administration on spatial memory performance, depression, and hippocampal cell density across two age groups in 50 Long-Evans rats. Spatial memory performance and depressive behavior were assessed using the object location task and forced swim task, respectively, and standard Nissl staining was used to assess hippocampal cell count in the granule layer of the dentate gyrus. It was hypothesized that animals that receive only antibiotics will have decreased spatial memory performance, increased depressive behavior, and decreased hippocampal cell count compared to controls and animals that received probiotics, but that this would be especially true for older animals. We also hypothesized that probiotic recovery of performance and hippocampal density would be greater in young than old rats. Baseline spatial memory performance and depressive behavior were compared to spatial memory performance and depressive behavior following antibiotic treatment and then again following probiotic treatment. Preliminary evidence showed that spatial memory performance was greater

at baseline compared to performance at final testing, particularly for rats that received antibiotics over rats that did not.

Disclosures: **A.G. Powell:** None. **R.M. Farmer:** None. **R.P. Ford:** None. **E.M. Gott:** None. **C.M. Gregg:** None. **A.R. Kniffin:** None. **S.M. Moseley:** None. **V.A. Wright:** None. **E.A. Zihal:** None. **M.L. Shoup-Knox:** None.

Poster

209. Brain Wellness and Aging: Systemic Factors and Brain Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 209.12/D25

Topic: C.01. Brain Wellness and Aging

Title: The effects of environmental enrichment and high-fat diet on aged mice

Authors: P. SONI, P. GHOTRA, T. SIMON, J. MAGANA, K. TIRADO-GONZALEZ, A. LEMUS, K. THOMASSIAN, J. MEISNER, T. UHLENDORF, *L. R. BANNER;
Biol., California State Univ. Northridge, Northridge, CA

Abstract: Human life expectancy is on the rise, therefore it is essential to understand the aging population and the link between aging and cognitive impairment. Normal aging is associated with atrophy and alterations in hippocampal plasticity among other changes. Coupled with this, more than one-third of the population in the United States is obese and this percentage is also projected to increase in the future. Increases in the number of cases of insulin-resistance and type-2 diabetes have paralleled the obesity rates. Studies have shown that obesity and type-2 diabetes significantly increases the risk of dementia and Alzheimer's disease and changes in hippocampal plasticity and spatial learning have been documented. It becomes important to understand the impact of obesity on the aging brain. Previous studies have shown that animals in an enriched environment show improvement in cognition, coordination, memory, and decrease tendency towards dementia. The purpose of our study is to evaluate the effect of environmental enrichment (EE) on aged male C57B16/J mice fed a long-term high-fat diet. Animals were randomly assigned to a standard mouse cage (which served as a control cage), with either a regular chow (RC) or high-fat diet (HFD) for 17 months. Animals on a HFD developed obesity and elevated blood glucose levels. At 18 months of age mice were further randomly assigned into HFD-Control (n=9), HFD-Enrichment (n=8). RC-Control (n=9), and RC-Enrichment (n=11) for two months. Animals in the enriched environment were placed in larger cages with a variety of toys that were changed on a weekly basis. The data collected was analyzed to determine what effect environmental enrichment had on blood glucose, weight, and performance on the elevated plus maze. A decrease in body weigh was seen for both groups in the enriched environment with the RC animals losing the most weight. Blood glucose levels were also slightly lowered in both enrichment groups. Preliminary results from the elevated plus maze indicates that aged animals

in the enrichment groups spent more time in the open arms and show a greater number of arm crossings compared to control groups. The results indicate that environmental enrichment is advantageous for aged obese mice.

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Poster

210. Alzheimer's Disease: Genetics

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 210.01/D26

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Gene expression reveals molecular insight for selective vulnerability in Alzheimer's disease

Authors: *O. GOZUTOK[1], S. PARK[1], E. OZALTIN[2], P. OZDINLER[1];
[1]Dept. of Neurol., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL; [2]Gebze Tech. Univ., Istanbul, Turkey

Abstract: The hippocampus is known for its complexity and function in learning, memory, and spatial navigation. The structure of hippocampus consists of several distinct regions, which include subiculum, the four cornu ammonis (CA) sectors, and the dentate gyrus. Each region is known for its unique function and connectivity. Interestingly, in diseases not all areas of the hippocampus is equally affected in patients. In an effort to build effective treatment strategies, we need to understand the molecular basis of their identification, connectivity and vulnerability in diseases. We *hypothesize* that even though all neurons have access to the same genetic material, not all neurons express the same genes. The choice they make and the genes they decide to express implement their identity, shape their connectivity and at times of diseases determine their vulnerability. Therefore, we think that gene expression profiles in distinct areas of the hippocampus help bring a mechanistic insight for the cellular events that are primarily important for their function, and could indeed be the causes of their vulnerability when perturbed. We investigated the expression profile of 2342 genes and grouped them based on the location they are primarily expressed. We found that 74 genes are primarily expressed in the dentate gyrus, 41 genes in the CA3 region, 29 in the CA1-2 region of the hippocampus. When the canonical pathways the proteins encoded by these genes are involved in are investigated by large-data management tool boxes, distinct cellular events are suggested to be more important in different parts of the hippocampus. In addition, some key proteins, with involvement in numerous cellular events, also became evident. These studies suggest that one of the reasons for selective vulnerability is their inability to maintain homeostasis for the canonical pathways that

are critically important for their function. Understanding those canonical pathways and key converging domains would help develop effective treatment strategies.

Disclosures: O. Gozutok: None. **S. Park:** None. **E. Ozaltin:** None. **P. Ozdinler:** None.

Poster

210. Alzheimer's Disease: Genetics

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 210.02/D27

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Introduction of deletion mutations associated with Alzheimer's disease into mouse genome

Authors: *K. SATO[1,2], H. SASAGURI[1], K. NAGATA[1,3], T. OHSHIMA[2], T. SAIDO[1];
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Abstract: *Presenilin1* (*PSEN1*) is the most frequent causal gene in inherited forms of Alzheimer's disease, in which more than 250 mutations including deletion of exon 9 (delta E9) are identified. However, the functional consequences of each mutation mostly remain uncovered *in vivo*. Here we introduced delta E9 mutation into the mouse *Psen1* gene utilizing CRISPR/Cas9 technology in order to explore the physiological effects of exon 9 deficiency in Psen1 protein *in vivo*. We designed two sgRNAs on intron 8 and intron 9, respectively to delete genomic region (around 800 bp) including exon 9 and the flanking introns. Both sgRNAs along with SaCas9 mRNA were injected into the cytoplasm of mouse zygotes via microinjection. CRISPR/Cas-mediated genome editing resulted in the efficient generation (33.8%) of the mutant mice harboring exon 9 deletion. Unexpectedly, one mouse (4.5%) showed two patterns of *Psen1* mRNA transcript covering the additional deletion of exon 8 (delta E8-E9) (90.1 %) and delta E9 (9.9%). It is noteworthy that exon 8 sequence of genomic DNA remained intact in this mouse, indicating exon 8 might be subjected to additional exon skipping by unknown mechanisms. All of the F1 mice in a heterozygous state displayed deletion of only exon 9 in mRNA, although the deletion mutation in genomic DNA of the delta E8-E9 mouse was transmittable to the F1. Although it has been reported that some of *Psen1* knock-in mice with point mutations exhibited embryonic lethality in a homozygous state, the delta E8-E9 mouse was viable and displayed no developmental defects except for tail deformity. These results demonstrate that CRISPR/Cas system allow the efficient generation of mutant animal models with disease-associated mutations, and suggest that the mechanism for the unexpected exon skipping and its effect for AD pathology should be further examined.

Disclosures: **K. Sato:** A. Employment/Salary (full or part-time):; junior research assistant in RIKEN (part-time). **H. Sasaguri:** A. Employment/Salary (full or part-time):; full. **K. Nagata:** None. **T. Ohshima:** A. Employment/Salary (full or part-time):; full. **T. Saido:** A. Employment/Salary (full or part-time):; full.

Poster

210. Alzheimer's Disease: Genetics

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 210.03/D28

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Contract Acadia Pharmaceuticals

Title: Characterizing the performance of an Alzheimer's disease model rat in the traveling salesperson task (TSP)

Authors: A. UNAPANTA[1], B. BUTUK[1], J. B. HALES[1], *R. BLASER[2];
[1]Psychological Sci., Univ. of San Diego, San Diego, CA; [2]Univ. of San Diego, San Diego, CA, CA

Abstract: Alzheimer's Disease (AD) is a form of dementia that negatively affects cognitive abilities and worsens over time. Two genes that play a role in neurodegeneration in humans are the APP (amyloid precursor protein) and PS1 (presenilin 1) genes, both involved in early-onset familial AD. We examined a transgenic rat model with mutations in these two genes to determine whether they experience cognitive decline with age that might be comparable to humans. The Traveling Salesman Problem (TSP) is a spatial task involving the selection of an efficient route between multiple targets, and it has been used to study spatial cognition and memory in both humans and non-human animals. Our goals were to 1) characterize the performance of transgenic AD rats in this task, 2) characterize the change in control rats' TSP performance with age and 3) determine if their trajectory differs from that of AD rats. We first familiarized and pre-trained groups of control and transgenic rats to human handling and the TSP task. Then, rats were tested in a series of target configurations which varied in the number and spatial arrangement of baited targets. Their behavior was video- recorded and later coded for measures including latency, revisits per target, and percent above optimal. Our results indicated that the transgenic AD rats were generally impaired on the TSP compared to controls. The study also suggests that there is a significant effect of gender on performance, with the effects of genotype significantly stronger in females than males. Overall, these findings provide further insight on how a naturalistic task such as the TSP can be useful when examining the cognitive deficits seen in Alzheimer's Disease.

Disclosures: **A. Unapanta:** None. **B. Butuk:** None. **J.B. Hales:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Acadia Pharmaceuticals. **R. Blaser:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Acadia Pharmaceuticals.

Poster

210. Alzheimer's Disease: Genetics

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 210.04/D29

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: The correlations among cognitive functions, postmortem brain pathologies and Alzheimer's disease related SNPs: A study based on Human Brain Bank in China

Authors: *Q. YANG;
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Abstract: Four Alzheimer's disease (AD)-related Single single nucleotide polymorphisms (SNPs) in Chinese were examined combined with test of clinical cognition and brain pathological analysis for the first time. The association of the clinical everyday cognitive states, the postmortem neuropathological changes and SNPs were analyzed in 130 human brains from the Chinese Academy of Medical Sciences/Peking Union Medical College (CAMS/PUMC) Human Brain Bank. The CAMS/PUMC Human Brain Bank currently has 233 brains which is one of the largest brain banks in China.
Pathological changes were evaluated with the “ABC” score following the guidelines of National Institute on Aging and the Alzheimer's Association (NIA-AA). We found four SNPs were related to the outcome of higher ABC score. *APOE ε4* (OR=4.483, p=0.004), *RS10498633* GT genotype (adjusted OR=2.380, p=0.028) and *RS2305421* GG genotype (adjusted OR=4.399, p=0.015). The result enhanced the understanding of the pathogenesis of Alzheimer's disease and distribution of the AD related SNPs in Chinese.
Keywords Human Brain bank; Alzheimer’s Disease; SNPs; *APOE ε4*; *ADAM10*; *SLC24A4*

Disclosures: **Q. Yang:** None.

Poster

210. Alzheimer's Disease: Genetics

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 210.05/D30

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Exploring the role of MIR-138 in the onset of Alzheimer's disease

Authors: *E. BOSCHER, C. GOUPIL, S. HÉBERT;
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Abstract: BACKGROUND: With the exception of rare mutations in *APP*, *PSEN1*, *PSEN2* genes causing autosomal dominant early-onset Alzheimer's disease EOAD (ADEOAD), little is known about the genetic factors underlying most (90-95%) EOAD cases. We recently identified copy number variations (CNVs) in microRNA (miR) genes that could contribute to risk for EOAD, including a duplication of the *MIR138-2* locus. Interestingly, miR-138 is increased in the cerebrospinal fluid of sporadic AD patients and associated with memory performance in the human population. **OBJECTIVE:** To better understand the role and impact of miR-138 on AD development. **METHODS:** We performed overexpression studies in neuronal cells and mice to reproduce artificially the increased *MIR138-2* gene dosage. For *in vivo* studies, miR-138 was delivered by intracerebroventricular injections at birth (P0) using an optimized adeno-associated virus (AAV). **RESULTS:** We will provide a detailed assessment of miR-138-dependent genes (e.g., Fermt2, Bace1, GSK3β and APP) and AD pathological markers (amyloid, tau) in the various biological models. On note, our *in vivo* model displays 1.5 to 2-fold overexpression of miR-138, as seen in EOAD. **CONCLUSIONS:** This study suggests that increased gene dosage of *MIR138-2* could contribute to AD risk, by regulating different biological pathways implicated in amyloid and tau metabolism, consistent with growing literature. *In vivo,* studies suggest an important impact of modest miR-138 overexpression in the mammalian brain. These results further strengthen the role of miRNA imbalance in the onset of AD.

Disclosures: E. Boscher: None. **C. Goupil:** None. **S. Hébert:** None.

Poster

210. Alzheimer's Disease: Genetics

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 210.06/D31

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSERC 210042

Title: The role of sigma-1-receptor in endoplasmic reticulum stress and Alzheimer's disease

Authors: *T. NGUYEN[1], K. FERGUSON[2], R. BERGERON[2];
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Abstract: Alzheimer's disease (AD) is a neurodegenerative disease that impairs cognitive functions. Despite decades of research there is currently no cure, and few therapeutic treatments are available. As such, novel therapeutic targets are desperately needed. AD has long been associated with an increase in aggregated Amyloid β (Aβ), and hyperphosphorylated tau. The cell has two main mechanisms to clear damaged, misfolded and aggregated proteins: autophagy and the ER-associated degradation system (ERAD). Perturbations in these pathways lead to ER stress, and subsequent cell death if not properly resolved. The Sigma-1 Receptor (Sig1R), a chaperone protein immersed in lipid rafts of the ER likely plays an important role in this process. Sig1R agonists have been shown to be neuroprotective and anti-amnesic in models of AD. Moreover, recent studies suggest that Sig1R plays a role in modulating ER stress, however its mechanism of action remains largely unknown. In our study, we use primary MEF cells derived from Wild-type (WT) and Sig1R Knock-out (KO) mice, and an acute Knock-down (KD) cell line, to examine the role of Sig1R in ER stress. It is known that ER stress triggers a signalling cascade called the Unfolded Protein Response (UPR). To examine the role of Sig1R in ER stress, we use western blot and RT-PCR to compare protein and mRNA transcript levels of downstream effectors of the UPR pathways (PERK, ATF6, and IRE1) following acute (dithiothreitol - DTT) and persistent (tunicamycin and thapsigargin) ER stress in all groups. Our results demonstrated that there was no difference in response to persistent stress between groups. Interestingly, the loss of Sig1R compromises the recovery from acute ER stress. Since DTT induces oxidative stress, we next examined the effect of oxidative stress on our models. Following treatment with an oxidative stressor, sodium-arsenite, we observed a significant transcript upregulation of ER stress markers, specifically in the PERK pathway, as well as autophagy markers, and a downregulation of an ERAD marker (EDEM) in KO and KD models compared to control. Additionally, we found overexpression of Sig1R attenuated the protein level increase in Nrf2 in HEK293 cells. Furthermore, preliminary results from Flow Cytometry suggest that Sig1R agonist has a protective effect against cell death caused by Aβ. Our data suggest that loss of Sig1R compromises autophagy and ERAD protein quality control mechanisms. These results could have important implications for Sig1R involvement in the clearance of aggregated proteins, leading to the dysfunctional recovery from ER stress.

Disclosures: **T. Nguyen:** None. **K. Ferguson:** None. **R. Bergeron:** None.

Poster

210. Alzheimer's Disease: Genetics

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 210.07/D32

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Cure Alzheimer's Fund
AFAR Glenn Foundation for Medical Research Postdoctoral Fellowships in Aging Research

Title: Rare functional angiotensin I converting enzyme genetic variants increase the risk of Alzheimer's disease

Authors: *L. K. CUDDY[1], D. PROKOPENKO[3], R. BRIMBERRY[2], E. CUNNINGHAM[2], P. SONG[2], D. PROCISSI[4], T. HANANIA[5], S. C. LEISER[5], R. E. TANZI[3], R. J. VASSAR[1]; [1]Dept. of Neurol., [2]Northwestern Univ., Chicago, IL; [3]Massachusetts Gen Hosp, Harvard Med. Sch., Charlestown, MA; [4]Northwestern Radiology, Chicago, IL; [5]Translational EEG, PsychoGenics, Paramus, NJ

Abstract: The angiotensin I converting enzyme (ACE1) gene (*ACE*) has been recently established as a genome-wide significant Alzheimer's disease (AD) risk gene. ACE1 is a zinc-dependent peptidase that is known for regulating blood pressure within the circulatory renin-angiotensin system (RAS). However, ACE1 has diverse physiological functions, including a role in in the peripheral immune response, and an intrinsic RAS has been found in the brain where ACE1 is expressed in neurons. ACE1 has been previously linked to AD by observations that midlife hypertension increases AD risk, ACE1 degrades Aβ42 and ACE1 protein level is upregulated in post-mortem AD brains. In this study, we identify rare functional *ACE* variants that could help identify new biologic insights. Here we gained access to a deep (>40x) whole genome sequencing family-based cohort from NIMH. The dataset consisted of 446 families with affected and unaffected siblings. We further focused on medium and high impact rare variants. Among rare variants with a potential functional impact we selected rs4980 (R1279Q). In this study, we investigate ACE1 R1279Q in knock-in ($ACE1^{KI/KI}$) mice with the cognate mutation in the murine *ACE* gene (R1284Q). We find that ACE1 R1284Q increases ACE1 protein level and activity in neurons and plasma, and is a toxic, gain-of-function mutation. $ACE1^{KI/KI}$ mice exhibited age-related neurodegeneration, EEG disruption and memory deficits, but had normal blood pressure and cerebrovascular function. In the brain, ACE1 R1284Q increased angiotensin II levels and caused selective neuron loss in the hippocampus. ACE1 R1284Q also increased the levels of circulating pro-inflammatory mediators known to be involved in the pathogenesis of autoimmune neuroinflammatory disorders. In 5XFAD $ACE1^{KI/KI}$ crosses, neurodegeneration was accelerated while Aβ42 level was unchanged. Finally, both central and peripherally-acting RAS

inhibitors prevented neurodegeneration in ACE1$^{KI/KI}$ mice, suggesting phenotypes in ACE1$^{KI/KI}$ mice are caused by a combined effect of central and peripheral, blood pressure-independent actions of ACE1 R1284Q. Our findings support of growing evidence that RAS-acting anti-hypertensive medications may have beneficial effects toward the treatment or prevention of AD, independently of their blood pressure lowering properties or effects on Aβ42 clearance. Our studies show for the first time a direct link between ACE1 function, neurodegeneration and AD.

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Poster

210. Alzheimer's Disease: Genetics

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 210.08/D33

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CONACYT S0008-2016-1 Nº 273182

Title: Evaluation of polymorphisms in the GSK3, MAPT, and HSP genes, as possible risk markers in refractory epilepsy and Alzheimer's disease

Authors: *V. CAMPOS-PEÑA[1], P. PICHARDO-ROJAS[2], G. RAMOS[3], D. GALVEZ[4], D. TORAL[5];
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Abstract: Alzheimer disease (AD) and temporal lobe epilepsy (TLE), are two common neurodegenerative diseases, that present hyperphosphorylation of tau protein, imbalance in the release and recapture of excitatory and inhibitory neurotransmitters, structural alterations of the neuronal cytoskeleton, synaptic loss, and neuroinflammation. These alterations are associated with the presence of hyperphosphorylated tau protein aggerates. The aim of this study was to determine whether the presence of single nucleotide polymorphisms (SNPs) present in *MAPT*, *HSP* and *GSK* genes are associated with temporal lobe epilepsy with and without hippocampal sclerosis (HS and TLE) in drug-resistant epilepsy and Alzheimer disease patients.
Seven polymorphisms were genotyped in 40 subjects with TLE, 66 with HS, 200 controls and 90 with AD. SNPs were genotyped using TaqMan probes (Applied Biosystems) with real-time PCR detection. The differences in allele, genotype and haplotype frequencies were analyzed and a Xi2 test was performed. Significant differences in genotypic frequencies between TLE and controls were found for rs222 and rs391 polymorphisms within HSP gene (T/T and C/C respectively, p<

0.05). In addition, rs146, rs242, and rs752 polymorphisms within MAPT gene (G/A, G/G and G/G respectively, $p< 0.05$) are mainly present in the TLE group.
In patients with HS, it was observed that the T/T and C/C genotypes, in rs222 and rs391 polymorphism of HSP were preferentially found in patients ($p < 0.05$)
Finally, in subjects with HS only the G/G genotype in rs242 polymorphism located in MAPT was preferably found in the TLE group vs. controls ($p < 0.05$)
The presence of certain polymorphisms in the genes that code for GSK3, HSP, and tau, could increase the risk of epilepsy in our population and these associations are presented differentially between the different types of epilepsy evaluated and AD. These alterations can also be associated with the degenerative process observed in this type of patients.

Disclosures: V. Campos-Peña: None. **P. Pichardo-rojas:** None. **G. Ramos:** None. **D. Galvez:** None. **D. Toral:** None.

Poster

210. Alzheimer's Disease: Genetics

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 210.09/D34

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01AG054180
AG057914
K01AG049164
R01AG059716
F31AG050357
P30AG038070
P50DA039841

Title: Cross-species analyses identify Dlgap2 as a mediator of age-related cognitive decline and Alzheimer's disease

Authors: *S. M. NEUNER[1,4], L. DUMITRESCU[5], L. ANDERSON[2], D. M. GATTI[1], A. R. OUELLETTE[3], E. MAHONY[5], J. A. BUBIER[1], G. CHURCHILL[1], L. PETERS[1], H.-S. YANG[6,7,8], C. REITZ[9,10,11], B. KUNKLE[12], C. C. WHITE[8,13], P. L. DE JAGER[13,8], J. A. SCHNEIDER[14], D. A. BENNETT[14], E. J. CHESLER[1], T. J. HOHMAN[5], C. C. KACZOROWSKI[1];
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Epigenomics Program, Broad Inst. of MIT and Harvard, Boston, MA; [9]Taub Inst. for Res. on Alzheimer's Dis., [10]Gertrude H. Sergievsky Ctr., [11]Neurol. and Epidemiology, Columbia Univ., New York, NY; [12]Miller Sch. of Med., Univ. of Miami, Miami, FL; [13]Ctr. for Translational and Computat. Neuroimmunology, Columbia Univ. Med. Ctr., New York, NY; [14]Rush Alzheimer's Dis. Center, Dept. of Neurol., Rush Univ. Med. Ctr., Chicago, IL

Abstract: Aging is the leading risk factor for a number of disorders, including dementias such as Alzheimer's disease (AD). The mechanisms that underlie healthy aging, particularly the cognitive aspects, remain poorly understood. The mouse represents a critical resource to identify genetic factors influencing complex traits, namely due to well-defined genetic backgrounds, well-controlled environmental conditions, and lower sample size requirements for genetic mapping than human populations. Here we perform a large-scale cross-sectional evaluation of cognitive performance in the genetically diverse Diversity Outbred (DO) population from 6 to 18 months of age. Quantitative trait loci (QTL) mapping identified genomic regions modifying working memory in DO mice. A single protein-coding gene, *Dlgap2*, emerged as the top positional candidate. As *Dlgap2* is a critical component of the synapse, we measured the number and type of spines present in the hippocampus of a subset of DO mice and found that by 18 months of age, there was a significant correlation between both the percentage of thin and stubby spines and working memory performance on the T-maze, providing a mechanistic link between Dlgap2 and cognitive function. In order to evaluate the translational relevance of this finding, expression of *DLGAP2* in postmortem brain tissue was used in combination with pathological and longitudinal cognitive measures from human patients, data from a previously published GWAS of AD, and new GWAS results in African Americans. We observed a robust association between *DLGAP2* and cognitive decline at the variant, gene expression, and methylation levels. Specifically, several variants in the *DLGAP2* region were associated with AD while lower cortical *DLGAP2* levels were observed in both MCI and AD relative to normal controls. Additionally, lower *DLGAP2* levels were associated with more plaques and tangles at autopsy as well as faster longitudinal cognitive decline. Finally, methylation in the *Dlgap2* region was associated with resilience to AD pathology. In summary, work here identifies *DLGAP2* as a mediator of cognitive decline in both mouse and humans and highlights the benefit of using genetically diverse mouse populations to inform human studies. Future studies will investigate the role of identified variants, precise molecular mechanisms involved in mediating cognitive decline, and utility of *DLGAP2* as a therapeutic target to promote healthy brain aging.

Disclosures: S.M. Neuner: None. **L. Dumitrescu:** None. **L. Anderson:** None. **D.M. Gatti:** None. **A.R. Ouellette:** None. **E. Mahony:** None. **J.A. Bubier:** None. **G. Churchill:** None. **L. Peters:** None. **H. Yang:** None. **C. Reitz:** None. **B. Kunkle:** None. **C.C. White:** None. **P.L. de Jager:** None. **J.A. Schneider:** None. **D.A. Bennett:** None. **E.J. Chesler:** None. **T.J. Hohman:** None. **C.C. Kaczorowski:** None.

Poster

210. Alzheimer's Disease: Genetics

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 210.10/D35

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA AG063287

Title: Genetic interaction of PICALM and APOE in Alzheimer's disease

Authors: *B. R. PLUIMER[1], X. W. XIE[1], Y. WU[1], J. ZENG[2], Z. ZHAO[3];
[2]Physiol. & Neurosci., [3]Physiol. & Biophysics, [1]USC, Los Angeles, CA

Abstract: Alzheimer's disease (AD) is the most common form of dementia in the elderly—characterized by progressive neurodegenerative conditions including amyloid plaque, neurofibrillary tangle formation, and cognitive impairment. Genetic inheritance is estimated to determine nearly 80% of the AD cases. In addition to the well-known familial mutations in *APP*, *PSEN1* and *PSEN2* genes found in early-onset AD cases, over 30 loci or genes are associated with sporadic late-onset AD (LOAD) as indicated by recent genome-wide association studies and whole exosome/genome sequencing projects. These studies have revealed two particularly influential genes—*APOE* and *PICALM*. *APOE* encodes the lipid carrier apolipoprotein E protein. Among its three major isoforms (ε2, ε3, and ε4), ε3 is the most common, ε4 is unarguably the strongest genetic risk factor for LOAD, and ε2 is the rarest but is protective for AD. These isoforms differentially affect molecular and cellular events that are important for amyloid-β (Aβ) metabolism and neurodegeneration. *PICALM* encodes the phosphatidylinositol binding clathrin assembly protein and is confirmed by GWAS studies as a major AD-associated gene. PICALM controls receptor internalization and subsequent intracellular trafficking of clathrin-coated vesicles. Furthermore, it plays key roles in mediating brain clearance of Aβ, regulating activities of β- and γ-secretases for Aβ production, mitigating Aβ toxicity in neurons, and promoting Tau clerance via autophagy. Interestingly, in population studies, a unique genetic interaction between *APOE* and *PICALM* in AD has been demonstrated; PICALM genotypes at multiple AD-associated sites confer risk—predominantly in ε4 carriers. And, the AD-risk PICALM rs3851179^{G} allele and *APOE* ε4 allele synergistically affect cortex volume and working memory function in AD patients. However, the mechanism underpinning this interaction in AD is still unknown.
By further investigating PICALM's interactome and functions in maintaining cell surface protein functions, we found that PICALM interacts with ABCA1 cholesterol and phospholipid transporter and that PICALM deficiency impaired APOE lipidation and reduced level of surface ABCA1. Therefore, PICALM may facilitate APOE lipidation and Aβ metabolism by controlling the function of ABCA1 transporter. Moreover, PICALM rs3851179^{G} and APOE ε4 alleles

adversely affect AD pathogenesis. In sum, our data provide new insights into the inheritability, etiology and pathogenesis of AD, and may serve as a foundation for future endeavors to target this interaction therapeutically for AD diagnosis and treatment.

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Poster

211. Tau: Preclinical and Clinical Pathology

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 211.01/D36

Topic: C.02. Alzheimer's Disease and Other Dementias

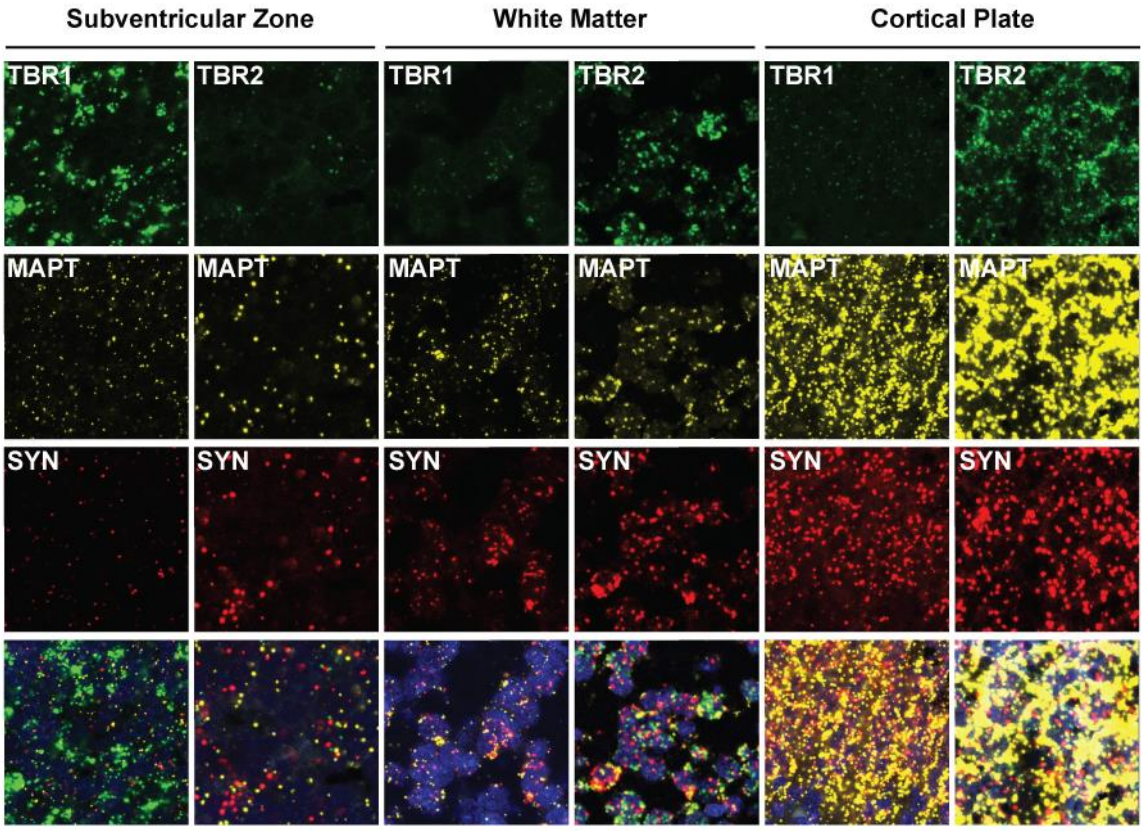
Support: NIH Grant UL1TR002537
NIH Grant R01AG054008
NIH Grant R01NS095252
DOD Grant 13267017
Williams Cannon Foundation Fellowship

Title: Temporal and regional mapping of tau expression in the developing human brain

Authors: K. L. FIOCK[1], M. E. SMALLEY[1], J. F. CRARY[2], ***M. M. HEFTI**[3];
[1]UNIVERSITY OF IOWA, Iowa City, IA; [2]Pathology, Icahn Sch. of Med. At Mount Sinai, New York, NY; [3]Univ. of Iowa, Iowa City, IA

Abstract: Although best known for its role in Alzheimer disease, we have previously shown that tau is expressed as early as the fifth post-conceptional week. It remains unclear however, when and in which cells tau expression begins during development. We thus sought to map tau mRNA and protein expression in the developing human brain at the cellular level using a combination of existing single cell RNA sequencing (sc-RNAseq) data, RNA in situ hybridization (RNAscope) and immunohistochemistry on human fetal brain tissue. Using sc-RNAseq, we found that tau mRNA expression begins in radial glia but increases dramatically as migrating neuronal precursors mature. Specifically TBR1+/TBR2-/SYN+ maturing neurons and TBR1-/TBR2-/SYN+ mature neurons showed significantly higher mRNA expression than GFAP+/NES+ radial glia or TBR1-/TBR2+/SYN+ intermediate progenitors. Using RNAscope, we found low levels of tau mRNA in subventricular zone radial glia and deep white matter intermediate progenitors, with a dramatic increase in more superficially located maturing neurons and mature neurons, using commercially available probes for the same markers identified by scRNA-seq (Fig 1). Interestingly, we found low levels of tau mRNA expression in fetal, but not neonatal astrocytes by RNAscope. We also found that by scRNAseq fetal interneurons show significantly lower levels of tau mRNA than fetal excitatory neurons. By total-tau (HT7) immunohistochemistry, the

germinal matrix and subventricular zone showed no protein expression, although mRNA was seen by both RNAscope and sc-RNAseq. Our results indicate that that tau expression increases with neuronal maturation and suggests that tau mRNA transcription may begin significantly earlier than protein expression. Further studies will be required to characterize tau expression in individual neuronal subtypes and in other areas of the developing brain, as well as to identify regulatory mechanisms triggering the onset of tau gene transcription and translation, which may also be potential therapeutic targets for neurodegenerative tauopathies.



Disclosures: **K.L. Fiock:** None. **M.E. Smalley:** None. **J.F. Crary:** None. **M.M. Hefti:** None.

Poster

211. Tau: Preclinical and Clinical Pathology

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Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 211.02/D37

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA PO1AG14449
NIH Grant R01AG043375
NIH Grant P30AG010161

Title: Oligomeric, hyperphosphorylated, and conformational tau pathology in the medial temporal lobe during the onset of Alzheimer's disease

Authors: *L. MAHADY[1], M. NADEEM[1], S. PEREZ[1], B. HE[1], M. MALEK-AHMADI[2], E. MUFSON[1];
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Abstract: The medial temporal lobe (MTL) connectome consists of the transentorhinal (TRC), entorhinal cortex (EC) and hippocampus (HP), which are associated with memory impairment in Alzheimer's disease (AD) and display neurofibrillary tangles (NFTs) first in the TRC, then EC, followed by the HP. Here we determined the evolution of NFTs using antibodies that mark oligomeric TOC1 and TNT, early (AT8) and late-stage tau marker (TauC3) within MTL tissue obtained from subjects who died with a premortem clinical diagnosis of no cognitive impairment (NCI), mild cognitive impairment (MCI), and AD. Counts of all four tau markers were compared between regions and within each region across clinical groups. Quantitation between regions revealed the number of HP TOC1 and TNT positive perikarya was significantly greater in MCI and AD compared to TRC and EC in NCI. Hippocampal TOC1 and TNT numbers were significantly greater in AD compared to NCI. Across regions the number of TOC1 and TNT positive perikarya was significantly greater in MCI and AD HP compared to NCI TEC and EC cortices, and TOC1 and TNT AD HP compared to NCI HP. AT8 numbers in the AD TEC and HP were significantly higher than those found in the TEC, EC and HP in NCI. TauC3 neuron numbers were significantly higher in the AD HP compared to the TEC, EC and HP in NCI. Quantitation within regions revealed that the numbers of TOC1, TNT and AT8 positive perikarya were significantly greater in the TRC and EC in MCI and AD compared to NCI. Analysis of HP subfields demonstrated that TOC1, TNT and AT8 positive perikarya were significantly higher in CA1 in AD compared to NCI. Counts of TOC1 and AT8 positive neurons in the subiculum revealed significantly more of each marker in MCI and AD compared to NCI. The number of TNT labeled neurons in the subiculum were higher in MCI compared to NCI, as well as in AD compared to MCI. In CA3, the number of TOC1 labeled neurons were significantly greater in AD compared to NCI and MCI, whereas TNT labeled neurons were significantly higher in MCI and AD compared to NCI. There was no significant difference in the number of AT8 or TauC3 between groups. Braak stage was positively correlated with all four tau markers in CA1; while AT8, TNT and TOC1 neuron number in the subiculum and EC positively correlated with Braak stage. In the TRC, only AT8 counts were significantly correlated with Braak stage. The present data demonstrate that the TEC and EC contain similar numbers of oligomeric, pretangle and late-stage tau pathology, whereas the hippocampus displays a greater number of each tau epitope across clinical groups. Together these data suggest that the evolution of NFT pathology occurs at an accelerated rate in the HP compared to the TEC and EC.

Disclosures: L. Mahady: None. **M. Nadeem:** None. **S. Perez:** None. **B. He:** None. **M. Malek-Ahmadi:** None. **E. Mufson:** None.

Poster

211. Tau: Preclinical and Clinical Pathology

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 211.03/D38

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG014449
NIH Grant AG053760
NIH Grant AG043375
NIH Grant AG107617
NIH Grant AG044372
NIH Grant NS082730
NIH Grant AG053581

Title: Dysregulation of neurotransmitter receptor gene expression is associated with tau oligomerization in cholinergic basal forebrain neurons during the progression of Alzheimer's disease

Authors: J. S. BECK[1], S. D. GINSBERG[2], N. M. KANAAN[1], C. T. TIERNAN[3], E. J. MUFSON[4], ***S. E. COUNTS**[1,5];
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Abstract: The microtubule-associated protein tau is the predominant protein found in neurofibrillary tangles (NFTs), which contribute to neuronal dysfunction and cognitive decline in Alzheimer's disease (AD) and other tauopathies. The exact role of tau in neurodegenerative diseases is not fully understood. However, accumulating evidence suggests that one of the toxic tau moieties may be soluble, oligomeric tau species. We have shown that tau oligomer accrual within vulnerable cholinergic basal forebrain (CBF) cortical projection neurons located within the nucleus basalis of Meynert (NbM) correlates with spatiotemporal patterns of CBF neuron loss in AD. To determine whether tau oligomerization is associated with specific molecular pathogenic alterations in these neurons, we compared gene expression profiles of laser capture microdissected NbM neurons singly immunostained for the pan-neurotrophin receptor $p75^{NTR}$, a well-established CBF neuronal marker, or dual-labeled for $p75^{NTR}$ and tau oligomeric complex 1 (TOC1), a marker of tau oligomers, using tissue obtained postmortem from Rush Religious Order Study participants who died with an antemortem clinical diagnosis of no cognitive impairment, mild cognitive impairment, or mild/moderate AD (n = 8-10/group). Preliminary quantitative analysis focused on TOC1-associated changes in classes of transcripts related to neurotransmitter receptor gene expression. We found a significant ~30-40% downregulation of

AMPA (*Gria2*) and NMDA (*Grin2A*, *Grin2B*, and *Grin2C*) glutamate receptor subunit transcripts, as well as a significant ~25-45% downregulation of genes encoding dopaminergic *Drd1* and *Drd2*, adrenergic *Adra1b* and *Adra2b*, and serotoninergic *Htr2b* G protein-coupled receptors, in $p75^{NTR}$/TOC1 co-labeled compared to $p75^{NTR}$ singly-reactive NbM neurons ($p < 0.05$). By contrast, the cholinergic *Chrna7* nicotinic receptor subunit was significantly upregulated by ~35% in dual-labeled NbM neurons ($p < 0.05$). Notably, TOC1-related transcript alterations were independent of diagnostic group, suggesting that expression level changes were related to neuronal tau oligomers and not clinical status. Hence, the accumulation of CBF neurons bearing tau oligomers may result in neurotransmitter-mediated dysregulation of cortical cholinergic signaling during the earliest stages of AD, which may be amenable to therapy.

Disclosures: **J.S. Beck:** None. **S.D. Ginsberg:** None. **N.M. Kanaan:** None. **C.T. Tiernan:** None. **E.J. Mufson:** None. **S.E. Counts:** None.

Poster

211. Tau: Preclinical and Clinical Pathology

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 211.04/D39

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH P01 AG014449
NIH R01 AG043375
NIH P30 AG010161

Title: Frontal cortex spliceosome deficits in early Alzheimer’s disease

Authors: *M. NADEEM, S. E. PEREZ, B. HE, L. MAHADY, E. J. MUFSON;
Barrow Neurolog. Inst., Phoenix, AZ

Abstract: Constitutive and alternative RNA splicing, a highly regulated process consisting of intronic/exonic sequence elimination required for the generation of mature mRNA, is performed within the spliceosome, a dynamic ribonucleoprotein (RNP) complex. Recent studies have demonstrated that alterations in splicing proteins are associated with neurofibrillary tangle and plaque pathology in AD, suggesting that disruption of RNA processing plays a key role in AD pathogenesis. However, virtually no studies have explored nuclear spliceosome protein changes within cortical neurons in the preclinical stages of AD. Therefore, we examined alterations the levels of several splicing proteins including RNA transcriptional polymerase II (POL) enzyme and phospho (p) POL, SR splicing factor 2 (SRSF2 or SC35), a protein also involved in alternative splicing of tau and amyloid precursor protein (APP) and the heterogeneous nuclear (hn) RNP A2B1, a protein implicated in trafficking and maturation of RNA within frontal cortex (FC) neurons in tissue obtained from individuals who died with an ante-mortem clinical

diagnosis of non-cognitive impairment (NCI), mild-cognitive impairment (MCI), mild/moderate AD (mAD) and severe AD (sAD) using immunoblot. FC western blot membranes showed three molecular weight bands at 35, 45 and 90 KDa for SRSF2. The other splicing proteins were visualized and analyzed at the predicted molecular weight. Immunoblot analysis revealed a significant reduction in 90kDa SRSF2 protein levels in the FC of sAD compared to NCI ($p<0.001$), while FC 35kDa and 45 kDa SRSF2 protein levels were unchanged across clinical groups. FC hnRNP A2B1 levels were also significantly reduced in sAD compared to MCI and NCI ($p<0.01$), but no differences were observed for this protein between MCI and NCI groups. FC POL levels were lower in mAD and in sAD compared to NCI clinical group ($p<0.001$), but no changes in FC pPOL levels were observed across clinical groups. We found that the levels of 90kDa SRSF2 were strongly positively correlated with POL levels across clinical groups ($r=0.68$, $p<0.001$), but not with pPOL, while hnRNP A2B1 values correlated strongly with POL and pPOL ($r=0.7$ and 0.55, $p<0.001$) but weaker with 35kDa SRSF2 values during disease progression. These data show that the spliceosome protein deficits detected in the FC occur in early and late clinical AD stages, suggesting a role of the spliceosome dysfunction in early cognitive decline in AD.

Disclosures: M. Nadeem: None. **S.E. Perez:** None. **B. He:** None. **L. Mahady:** None. **E.J. Mufson:** None.

Poster

211. Tau: Preclinical and Clinical Pathology

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 211.05/D40

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Matrix metalloproteinase-9 and tau are related to white matter hyperintensity burden in individuals with Alzheimer’s disease

Authors: *K. K. LAING[1], I. C. TURNEY[1], H. W. MORENO[2], F. C. BARONE[3], A. M. BRICKMAN[1];
[1]Columbia Univ., New York, NY; [2]Dept Neurol & Physiol, SUNY Downstate, Brooklyn, NY; [3]Neurol., SUNY Downstate Med. Ctr., Brooklyn, NY

Abstract: Objective: Small vessel cerebrovascular disease (CVD), visualized as white matter hyperintensities (WMH) on T2-weighted magnetic resonance imaging (MRI), contributes to the clinical presentation and possibly the pathogenesis of Alzheimer’s disease (AD). In a previous study, we demonstrated that plasma tau levels and WMH volumes are correlated among individuals with AD. However, the pathway through which CVD and plasma tau levels are associated is poorly understood. Matrix metalloproteinases (MMPs), a family of zinc- and calcium-dependent enzymes involved in degrading and remodeling of the extracellular matrix,

have been implicated in the pathophysiology of cerebral ischemia, blood-brain barrier disruption, and neuroinflammation. We hypothesized that MMP concentrations are correlated with plasma tau pathology as a direct result of ischemic injury damage. The purpose of this study was to examine the associations of MMP9 plasma concentration, plasma tau levels, and their interaction, on WMH in individuals with AD.
Participants and Methods: We obtained plasma total-tau, plasma MMP9 concentrations, cerebral WMH, demographic and diagnostic variables for 235 participants from the Alzheimer's Disease Neuroimaging Initiative (ADNI). Participants were classified clinically as AD (n = 66), mild cognitive impairment (MCI; n = 142), or cognitively normal controls (NC; n = 45). In a mixed design general linear model, we investigated the association of diagnosis, MMP9, tau, and their interaction with WMH.
Results: Elevated plasma MMP9 and plasma tau measurements were associated with larger volumes of WMH (main effects of MMP9 and tau: $F=8.2$, $p=0.005$, $F=8.8$, $p=0.003$). Individuals with elevated concentrations of both tau and MMP9 also had greater WMH burden (MMP9 x tau interaction, $F = 11.01$, $p=0.001$), particularly in the AD subgroup (Diagnosis x MMP9 x tau interaction, $F=14.8$, $p<0.001$). Age was also associated with increased WMH ($p=0.01$).
Conclusion: These findings demonstrate that higher plasma concentrations of MMP9 and tau are independently and interactively associated with WMH burden, particularly among individuals with AD. One possible interpretation is that plasma MMP9, as a marker of extracellular matrix and endothelial cell decomposition, contributes to tau pathology and exacerbates CVD. Another non-mutually exclusive possibility is that elevated concentrations of MMP9 are a consequence of CVD and may induce tau pathology.

Disclosures: K.K. Laing: None. **I.C. Turney:** None. **H.W. Moreno:** None. **F.C. Barone:** None. **A.M. Brickman:** None.

Poster

211. Tau: Preclinical and Clinical Pathology

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 211.06/D41

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01AG054008
NIH Grant RO1NS107396

Title: Rna binding proteins in tauopathy

Authors: *R. SPEAR[1], M. TAKACS[2], K. FUSHIMI[1], J. WU[3], W. A. MCGEE[4], A. KAR[5]; [1]Neurol., [2]Neurosci., Northwestern Univ., Chicago, IL; [3]Neurology, Cancer Ctr, CGM, Northwestern Univ. Sch. Med., Chicago, IL; [4]Neurol., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL; [5]SNB, LMB, Natl. Inst. of Mental Hlth., NIH, Bethesda, MD

Abstract: Alzheimer's disease (AD) and related neurodegenerative diseases are becoming a leading public health challenge. The presence of tau protein-containing neurofibrillary tangles is a major neuropathological hallmark of AD and related tauopathy. Expression and function of the human tau gene is under complex transcriptional and post-transcriptional regulation. Although RNA binding proteins (RBPs) are emerging as critical players in a range of neurodegenerative diseases, little is known about the RBP networks that control the balanced expression of the human tau gene or other AD-associated genes. We have begun to systematically investigate human RBP genes in regulating tau gene expression and have revealed RbFox2 (Feminizing locus on X gene family member 2) as a critical regulator of tau exon 10 alternative splicing. Microtubule-associated protein Tau plays an important role in microtubule stabilization, neuronal function and survival. In the human brain, alternative splicing of exons 2, 3, and 10 in the tau pre-mRNA generates Tau protein isoforms with distinct functional properties. Alternative splicing of tau exon10 is tightly regulated in the adult human brain. Mutations in the tau gene that result in aberrant tau exon10 splicing have been associated with tauopathies. Previous studies have reported the presence of a stem-loop structure at the exon10-intron10 junction that regulates tau exon10 splicing. However, little is known about the trans-acting factors that interact and regulate this stem-loop structure. Here, we report identification of RbFox2 as a protein interacting with tau exon10 stem-loop region using RNA affinity purification-coupled mass-spectrometry strategy. RbFox2 promotes tau exon10 exclusion by interacting with a non-canonical binding site located downstream of exon10 stem-loop structure. Results of RNaseH cleavage assay suggest that RbFox2 mediates the stabilization of the exon10 stem-loop via its interaction with the non-canonical binding site. Taken together, our data uncover previously unknown function of RbFox2 in tau exon10 splicing regulation and reveals a non-canonical functional binding site for the RbFox2 protein. Our study has uncovered function RbFox2 in maintaining normal function of the human brain. Implications of our findings will be discussed at the meeting.
Supported by NIH (R01AG054008, RO1NS107396)

Disclosures: **R. Spear:** None. **M. Takacs:** None. **K. Fushimi:** None. **J. Wu:** None. **W.A. McGee:** None. **A. Kar:** None.

Poster

211. Tau: Preclinical and Clinical Pathology

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 211.07/D42

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AMED JP17dm0207014h0004

Title: Chronic neuronal activation by optogenetics enhances seed-induced Alzheimer's disease like tau pathology spreading

Authors: *I. NISHIDA[1], K. YAMADA[1], T. WAKABAYASHI[1,2], T. IWATSUBO[1];
[1]Neuropathology, Grad. Sch. of Med., [2]Innovative Dementia Prevention, Grad. Sch. of Med., The Univ. of Tokyo, Tokyo, Japan

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disease pathologically characterized by the intracellular accumulation of neurofibrillary tangles composed of filamentous tau protein. Tau pathology characteristically manifests in anatomically connected brain regions; tau deposition initiates in the entorhinal cortex, spreads into the hippocampus and then cerebral neocortices in AD brains.
Recent studies have suggested prion-like propagation of aggregated tau via extracellular spaces as one of the mechanisms that accounts for the hierarchical progression of tau pathology in AD. However, it remains unknown how tau propagates along with neuronal connections. Based on the observations that tau release occurs in a neuronal activity-dependent manner, here we hypothesized that neuronal activity influences propagation of tau pathology along with neuronal connections, specifically from entorhinal cortex to hippocampus.
We utilized a seed-induced model of tau propagation, in which recombinant tau fibrils were microinjected into the entorhinal cortex of young tau transgenic (PS19) mice overexpressing P301S mutant tau prior to the accumulation of tau pathology. We observed that ten weeks after microinjection, tau pathology was induced in neurons of the entorhinal cortex as well as in the ipsilateral hippocampus.
Next, we conducted chronic stimulation of the entorhinal cortex using optogenetics in these mice to examine whether neuronal activity affects tau propagation. To this end, we utilized stabilized step function opsin (SSFO), which can depolarize neurons for prolonged periods. AAV1 vectors encoding SSFO were injected in the entorhinal cortex of PS19 mice simultaneously with tau fibrils. Four weeks after the viral injection, projection from entorhinal cortex to hippocampus was activated by blue light stimulation once a day for 4 weeks.
We found that tau pathology was markedly enhanced not only in hippocampus but also in entorhinal cortex of the PS19 mice infected with AAV1-SSFO compared to control PS19 mice. Biochemical experiments also showed the marked accumulation of insoluble tau in PS19 mice infected with AAV1-SSFO.
The current results suggest that chronic neuronal activation augmented propagation of tau pathology along the hippocampal perforant pathway. The detailed cellular mechanism whereby neuronal activity enhanced the transmission of tau pathology should further be investigated.

Disclosures: I. Nishida: None. **K. Yamada:** None. **T. Wakabayashi:** None. **T. Iwatsubo:** None.

Poster

211. Tau: Preclinical and Clinical Pathology

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 211.08/D43

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: MOST Grant 106 - 2311 - B - 007 - 008 - MY3

Title: Truncation of tau at Asp421 ameliorated full-length human tau's toxicity under hyperphosphorylation condition

Authors: *H. CHI, Mr[1], T.-K. SANG[2];
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Abstract: Tauopathy represent a group of neuropathological conditions including Alzheimer's diseases and various parkinsonisms, which are all related to the abnormal behavior of tau protein after it dissociated from microtubule. In biopsy samples, it is commonly found the tau proteins form aggregations and being hyperphosphorylated, further analysis revealed that the core of the aggregation contains truncated tau proteins. Therefore, it has been long suspected tau protein posttranslational modifications including phosphorylation could have a huge impact on its toxicity through modulating its binding affinity to microtubule and to other proteins. To study this hypothesis, we generated a panel of different tau isoforms, taking tau truncation and phosphorylation status into consideration. 14 phosphorylation sites and one truncation site, which is most commonly found in biopsy samples were taken into evaluation and a panel of transgenic flies were generated to serve this purpose. First we evaluated the toxicity of the different isoforms in fly eye and surprisingly we found that both hyperphosphorylation and hypophosphorylation could enhance the toxicity of the protein as both conditions could enhance the fly neuropathological phenotypes including rough eye induction and disorientation of both posterior and anterior photoreceptors. Further analysis in the fly brain not only confirmed the preliminary findings in the fly eyes but also pointing out that truncation at Asp421 could significantly ameliorated tau toxicity under the hyperphosphorylation condition. As compared to the full length hyperphosphorylated tau, truncation at Asp421 ameliorated the brain pathological changes, including some brain pathology hallmarks including axon dilation, hirano body-like struncture formation, while also changed the distribution of tau protein within the neuron, neuronal membrane permeability and fly mobility. These findings indicate that a balance of tau phosphorylation/non-phosphorylation status is important for it to be less toxic and the truncation at the residue 421 could ameliorate the tau toxicity instead of enhancing it.

Disclosures: H. Chi: None. **T. Sang:** None.

Poster

211. Tau: Preclinical and Clinical Pathology

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 211.09/D44

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01AG022102-15

Title: Short- and long-term effects of acute hyperphosphorylation of tau in transgenic mouse models of Alzheimer's disease

Authors: *J. D. EUN[1,2,3], H. JIMENEZ[1], L. ADRIEN[1], P. DAVIES[1,2];
[1]Litwin-Zucker Ctr. for Alzheimer's Dis. & Memory Disorders, Feinstein Inst. for Med. Res., Manhasset, NY; [2]Pathology and Neurosci., Donald and Barbara Zucker Sch. of Med. at Hofstra/Northwell, Manhasset, NY; [3]Donald & Barbara Zucker Sch. of Med. at Hofstra/Northwell, Hempstead, NY

Abstract: Prolonged anesthesia in older people is sometimes associated with cognitive decline, although the mechanisms responsible for this phenomenon are obscure. The tau aggregates that characterize Alzheimer's disease and other tauopathies are abnormally phosphorylated, conformationally altered forms of the protein that accumulate in neurons. Anesthesia without temperature control acutely induces large increases in phosphorylation of tau, but the long-term effects of this hyperphosphorylation, including the development of later tau pathology, are unclear. Anesthesia provides the opportunity to study the mechanisms that underlie the association between hyperphosphorylation and subsequent development of tau pathology so that we can identify potential targets for disease-modifying therapies. We exposed two different human tau transgenic mice to isoflurane for two hours and evaluated the mice immediately or months after anesthesia. In addition to ELISA and immunocytochemistry, we assessed the gene expression changes in the brain in order to analyze their association with the levels of hyperphosphorylation and tau pathology. We found that in both P301L and COMTKO/P301L mice, the levels of abnormal phosphorylation measured by CP13 and PHF1 in the soluble fractions of cortex and hippocampus were much greater in the anesthesia group than that of the controls, but 24 hours after anesthesia, the levels of phosphorylation in the anesthesia group were not different from that of the controls. One month after anesthesia, the level of phosphorylation in the soluble fractions was similar between the anesthesia group and controls, but we found the the anesthesia group to have an elevated level of RZ3 in the insoluble fraction of cortex compared to that of the controls. We also used a conformational antibody MC1 to evaluate the development of tau pathology at one month post-anesthesia. Differential expression analysis suggests possible downstream effects of acute abnormal phosphorylation that might be associated the development of tau pathology.

Disclosures: **J.D. Eun:** None. **H. Jimenez:** None. **L. Adrien:** None. **P. Davies:** None.

Poster

211. Tau: Preclinical and Clinical Pathology

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 211.10/D45

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Fulbright Research Grant - Fulbright Commission of Spain

Title: Pathological gradients along the longitudinal axis of the human hippocampus in Alzheimer's disease

Authors: *K. S. BRESS[1], A. RÁBANO[1], B. STRANGE[2];
[1]Dept. of Pathology, [2]Dept. of Neuroimaging, Fundación Reina Sofía - Ctr. de Investigación de Enfermedades Neurológicas (CIEN), Madrid, Spain

Abstract: The hippocampus plays a critical role in a variety of cognitive functions, including learning and memory. Hippocampal vulnerability to the formation of neurofibrillary tangles and beta-amyloid protein deposition is a key pathological feature of Alzheimer's disease (AD). Resultant atrophy of the hippocampus, and disruption of its functions, is a core trait of AD-related dementia. Importantly, the human hippocampus is organized along an anterior-posterior longitudinal axis. Considering the distinct functional properties which are attributed to the anterior and posterior levels, differential vulnerability to pathological protein aggregation along the hippocampal axis is relevant to understanding both presentation and progression of AD-related neurodegeneration. In this study, we hypothesized that AD-type tau and beta-amyloid proteins would be distributed in a gradient-like manner along the axis of the hippocampus, with anterior hippocampus (HCA) showing a significantly lesser load than the hippocampal body (HCB) and tail (HCT). We also hypothesized the the distribution pattern of disease proteins within hippocampal substructures (CA1, CA2, CA3, and dentate gyrus) would be conserved across the three anatomical levels of the hippocampus. Samples of human hippocampal tissue were obtained from the Banco de Tejidos CIEN [BT-CIEN n=420]. We randomly selected n=10 cases with a primary neuropathological diagnosis of AD (Braak stage > 4), excluding those with high levels of hippocampal atrophy, hippocampal sclerosis, or identified concurrent pathologies. Blocks of hippocampal tissue were collected from each anatomical level of the hippocampus, defining HCA as tissue anterior to the uncus and the HCB as tissue anterior to the lateral geniculate nucleus. Immunohistochemistry for tau and beta-amyloid proteins was then performed on paraffin slices. We found that the load of NFTs, but not beta-amyloid plaques, was significantly different between HCA and HCP. Tangle and plaque distribution within hippocampal substructures presented similarly across the two levels. We are currently quantifying the load of NFTs and beta-amyloid plaques in samples of HCT tissue. These results

will address the relationship between functional organization of the human hippocampus and the presentation of AD, exploring the clinical implications of pathological gradients along the anterior-posterior longitudinal axis.

Disclosures: K.S. Bress: None. **A. Rábano:** None. **B. Strange:** None.

Poster

211. Tau: Preclinical and Clinical Pathology

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 211.11/D46

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Tau pathology reduction with SM07883, a novel, potent, and selective oral DYRK1A inhibitor

Authors: *C. LAI, K. DUONG-POLK, B. GÜNER, S. HABROUN, S. D. ANDERSON, B. MELCHIOR;
Samumed, LLC, San Diego, CA

Abstract: Dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A overexpression in Alzheimer's disease (AD) is correlated with tau hyperphosphorylation and formation of neurofibrillary tangles (NFTs). This study assessed the potential of SM07883, an oral DYRK1A inhibitor, to inhibit tau hyperphosphorylation, aggregation, NFT formation, and associated functional phenotypes in mouse models. To assess impact on neuroinflammation, glial activation was also analyzed.
SM07883 potency was evaluated in kinase panels and tau phosphorylation (pTau) was measured in cell-based assays. To assess long-term efficacy, pTau and aggregated tau were biochemically quantified in brain stems and spinal cords lysates from 10-month-old JNPL3 mice (P301L human tau overexpression mutation in the hindbrain and spinal cord resulting in locomotor impairment) treated with SM07883 (3mg/kg, QD, n=19; 10mg/kg QD, n=13; or QoD, n=19; 3 months) or vehicle (n=20). Intracellular tau inclusions were quantified by immunostaining. Gliosis was assessed using glial fibrillary associated protein (GFAP) and Iba1 staining. A wire hanging test was used to assess motor coordination at the beginning and the end of treatment. SM07883 selectively and potently inhibited DYRK1A kinase activity (IC_{50}=2nM). In cells, SM07883 reduced pTau, especially at the DYRK1A-relevant Threonine 212 site (EC_{50}=16nM). JNPL3 mice treated with SM07883 demonstrated significant ($p<0.05$) reductions in pTau at multiple sites, aggregated forms of tau, and tau-positive intracellular inclusion staining compared to vehicle. Reduced gliosis was confirmed by ELISA ($p<0.001$) and Iba1 stains showed a significant decrease in microglial cell count compared to vehicle ($p<0.001$). SM07883 was well tolerated and treated mice had a positive net weight gain over the course of the study (1.3g +/- 0.57). SM07883-treated mice showed a reduction of the clinical signs. Motor function in the wire

hanging test was significantly improved in SM07883-treated JNPL3 mice compared to vehicle (p=0.048).
SM07883, a selective, potent, and oral DYRK1A inhibitor currently being tested in a clinical trial, significantly reduced tau phosphorylation, pathological tau overexpression and associated neuroinflammation, and improved functional endpoints compared to vehicle. SM07883 has potential as a treatment for chronic tauopathies such as AD.

Disclosures: **C. Lai:** A. Employment/Salary (full or part-time):; full-time. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); stock options. **K. Duong-Polk:** A. Employment/Salary (full or part-time):; full-time. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); stock options. **B. Güner:** A. Employment/Salary (full or part-time):; full-time. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); stock options. **S. Habroun:** A. Employment/Salary (full or part-time):; full-time. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); stock options. **S.D. Anderson:** A. Employment/Salary (full or part-time):; full-time. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); stock options. **B. Melchior:** A. Employment/Salary (full or part-time):; full-time. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); stock options.

Poster

212. Alzheimer's Disease and Therapeutic Strategies I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 212.01/E1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant U01 AG052460

Title: Prostaglandin receptor EP2 antagonism reduces neuroinflammation in 5XFAD mouse model of Alzheimer’s disease

Authors: *A. BANIK, R. AMARADHI, W. WANG, N. H. VARVEL, R. J. DINGLEDINE, T. GANESH;
Dept. of Pharmacol. and Chem. Biol., Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Cyclooxygenase-2 (COX-2), a key enzyme responsible for prostaglandin synthesis, is induced in Alzheimer’s disease (AD) brains at the early Braak-stages, and could be involved in AD pathogenesis. Although COX-2 inhibitors have been approved as anti-inflammatory drugs

for peripheral conditions, their adverse cardiovascular effects have dampened future therapeutic applications in CNS disease areas. Thus, we examined downstream prostanoid receptor signaling to ameliorate the COX-2 mediated neuroinflammation in AD brains. Our previous studies highlighted the role of EP2 receptors in mediating much of the pro-inflammatory effects of COX-2 in rodents that experienced status epilepticus. In this study, we examined the effect of chronic treatment with a potent and selective EP2 antagonist, TG11-77.HCl, in the 5XFAD transgenic mouse model of AD. First, we characterized the potency, selectivity and anti-inflammatory properties of TG11-77.HCl in glial cells in culture. Then, 5XFAD mice and their non-transgenic littermates were treated for two months with TG11-77.HCl 100mg/kg/daily in drinking water. Mice were administered 0.5 mg/kg lipopolysaccharide (LPS) by intraperitoneal injection once a week to induce a low-level brain inflammation. Complete blood count (CBC) analysis revealed an inflammatory effect of LPS in WBC, RBC and platelet distribution, which was not altered by TG11-77.HCl treatment. TG11-77.HCl did not alter body weight throughout the treatment regime. The brain tissue analysis revealed that in female mice the mRNA level of proinflammatory mediators (IL-1β, TNFα, IL-6, CCl2, CXCL10) and glial markers (IBA1, GFAP, CD11b, S110B) were significantly reduced by TG11-77.HCl, whereas in male brains this effect was not found. There was no effect on the overall number of amyloid plaques or area covered by them in different regions of brain. Taken together our findings suggest a therapeutic effect of EP2 antagonism in ameliorating chronic neuroinflammation in AD brain. Investigations are underway to elucidate the underlying mechanisms involving in this anti-inflammatory effect.

Disclosures: **A. Banik:** None. **R. Amaradhi:** None. **W. Wang:** None. **N.H. Varvel:** None. **R.J. Dingledine:** None. **T. Ganesh:** None.

Poster

212. Alzheimer's Disease and Therapeutic Strategies I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 212.02/E2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant P30 NS47466

Title: The effects of treatment with tandem D-peptides in AD model mice

Authors: *T. VAN GROEN[1], I. KADISH[2], D. WILLBOLD[3];
[1]Univ. Alabama-Birmingham, Birmingham, AL; [2]Dept Cell, Developmental and Integrative Bio, Univ. of Alabama Birmingham, Birmingham, AL; [3]Forschungszentrum Jülich Gmbh, Jülich, Germany

Abstract: Alzheimer’s disease is a neurodegenerative disease whose causative factors are complex and not yet fully understood. It is thought that overproduction and/or decreased

clearance of amyloid beta monomers fuel the development of toxic amyloid beta oligomers that initiate various downstream events that ultimately lead to the cognitive decline associated with Alzheimer's disease. Our lab previously found that treatments with the amyloid beta 42 monomer stabilizing all-D-peptides D3 and RD2 improve cognition in an AD mouse model, most probably by direct elimination of toxic amyloid beta oligomers. The amyloid beta binding properties differ slightly between these two all-D-peptides and RD2 is significantly more efficient in oligomer elimination. This study tests the hypothesis that homomeric and heteromeric head-to-tail tandem versions of D3 and RD2 are more efficient on cognition improvement. Mice were treated with the tandem all-Dpeptides D3D3, RD2D3 or RD2RD2 or saline control, delivered via Alzet minipumps surgically inserted beneath the peritoneal layers, for four weeks. Behavioral tests including the open field test, the zero maze test, and the water maze were performed in order to determine stress or anxiety levels, and study spatial learning and memory in these mice. Immunohistochemistry was performed to identify changes in levels of inflammation and amyloid-beta deposition. While treatment with D3D3 improved cognition, treatment with RD2RD2 had no effect on cognition. Even more surprisingly, RD2D3 negatively affected cognition. Also, neither D3D3, RD2D3 or RD2RD2 reduced amyloid-beta deposits or amyloid pathology, similar to the non-tandem compounds. The fact that one tandem all-D-peptide improved cognition in Alzheimer's disease model mice is encouraging. However, our hypothesis that tandem peptides in general would be more efficient than their single components was shown to be incorrect. We speculate the potential of the tandem all-D-peptides to interact with more than one amyloid beta monomer may increase the risk that amyloid beta assemblies be stabilized in their presence.

Disclosures: T. van Groen: None. **I. Kadish:** None. **D. Willbold:** None.

Poster

212. Alzheimer's Disease and Therapeutic Strategies I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 212.03/E3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Sigrid Juselius Foundation
Olav Thon Foundation

Title: Response of spike-wave discharges in aged APP/PS1 Alzheimer model mice to antiepileptic, metabolic and cholinergic drugs

Authors: *N. JIN, S. ZIYATDINOVA, I. GUREVICIENE, H. TANILA;
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Abstract: Epileptic nonconvulsive spike-wave discharges (SWDs) are commonly seen in amyloid plaque bearing transgenic mice but only rarely in their wild-type littermates. To shed light on potential treatment mechanisms of SWDs, we assessed the effect of prototypic antiepileptic drugs (ethosuximide and levetiracetam), donepezil as the typical Alzheimer drug and atropine as an antagonistic effect, $GABA_B$ antagonist CGP-35348, and alternate energy substrates beta-hydroxybyturate (BHB), puryvate and lactate on the occurrence of SWDs in aged APPswe/PS1dE9 mice. All agents were administered by single intraperitoneal injections at doses earlier documented to be effective and response was assessed by recording 3 h of video-EEG. Atropine significantly increased locomotor activity of APP/PS1 mice. Ethosuximide at 200 mg/kg effectively suppressed SWDs while levetiracetam at 75 mg/kg had no effect. Unexpectantly, donepezil at 0.3 mg/kg had no effect on SWDs while atropine at 25 mg/kg dramatically reduced their occurrence, but also resulted in EEG slowing and reduced immobility time. CGP-35348 at 100 mg/kg had no effect on SWDs. Lactate at 1 g/kg increased the occurrence of SWDs while BHB and pyruvate at the same dose was ineffective. These findings call for re-evaluation of some prevailing theories on neural circuit alternations that underlie SWD generation and show the utility of APP/PS1 mice for testing potential new treatments for nonconvulsive epileptic activity related to Alzheimer brain pathology.

Disclosures: **N. Jin:** None. **S. Ziyatdinova:** None. **I. Gureviciene:** None. **H. Tanila:** None.

Poster

212. Alzheimer's Disease and Therapeutic Strategies I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 212.04/E4

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Discovery of potent, brain penetrant PROTAC® degrader molecules that significantly reduce pathologic tau protein species in *in vivo* models of tauopathies

Authors: *J. T. CHANDLER[1], J. J. FLANAGAN[1], M. BERLIN[1], G. CADELINA[1], Y. HUANG[1], S. KEENAN[1], J. PIZZANO[1], M. BOOKBINDER[1], A. P. CREW[1], C. M. CREWS[2], I. TAYLOR[1], J. HOUSTON[1], A. CACACE[1];
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Abstract: Alzheimer’s Disease (AD) is the most frequent tauopathy, characterized by hyperphosphorylated neurofibrillary tangles (NFTs) consisting of the microtubule-associated protein, tau (MAPT). Our strategy is to hijack the ubiquitin-proteasome system to specifically target pathogenic tau aggregates for elimination using the PROteolysis TArgeting Chimera (PROTAC®) technology. Here we describe peripherally-administered, tau-directed PROTAC® molecules that degrade pathologic tau aggregates in the mouse brain. These tau PROTAC® bifunctional compounds degrade approximately 98% of pathologic aggregates (defined by

hyperphosphorylated sarkosyl-insoluble tau) in models containing FTDP-17 mutations (eg. P301L). Tau PROTAC®-degraders have achieved half-maximal degradation concentrations (DC_{50}) <10nM when tested in an *in-vitro* human P301L tau model system. Moreover, competition experiments demonstrate that the degradation activity of the tau PROTAC® degraders are dependent on binding to both tau and to the hijacked E3 ubiquitin ligase. Following peripheral administration of 3 mg/kg tau PROTAC® molecules in homozygous JNPL3 tauopathy mice, robust and uniform bioavailability is observed across the brain. Similarly, degradation of hyperphosphorylated sarkosyl-insoluble tau species occurs throughout the brain including the hippocampus. Additional studies to assess prevention of both intracellular dysfunction and extracellular pathologic propagation are ongoing. These data suggest that tau PROTAC® bifunctional compounds may represent novel therapeutics for the treatment of tauopathies.

Disclosures: J.T. Chandler: None. **J.J. Flanagan:** None. **M. Berlin:** None. **G. Cadelina:** None. **Y. Huang:** None. **S. Keenan:** None. **J. Pizzano:** None. **M. Bookbinder:** None. **A.P. Crew:** None. **C.M. Crews:** None. **I. Taylor:** None. **J. Houston:** None. **A. Cacace:** None.

Poster

212. Alzheimer's Disease and Therapeutic Strategies I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 212.05/E5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R56AG05755501
NIH Grant 2R25GM060665

Title: Cytokine regulation via the MyD88-dependent toll-like receptor signaling pathway unveils neuroprotective mechanisms in the transgenic rat model of Alzheimer's disease

Authors: *G. OLIVEROS[1], C. WALLACE[1], R. SHRESTHA[1], S. YUMISEBA[1], S. ALOE[1], L. XIE[2], P. A. SERRANO[3], P. ROCKWELL[1], M. E. FIGUEIREDO-PEREIRA[1];
[1]Dept. of Biol. Sci., [2]Dept. of Computer Sci., [3]Dept. of Psychology, CUNY Hunter Col., New York, NY

Abstract: Amyloid-beta (Aβ) buildup, neurofibrillary tangles containing Tau, and increases in neuroinflammation are involved in Alzheimer’s Disease (AD). Our studies focus on the Toll-Like Receptor (TLR) signaling pathway, which regulates the expression of cytokines, such as interleukins 1β and 6 (IL-1β, IL-6). The TLR pathway includes an MyD88-dependent signaling cascade, which leads to cytokine expression and secretion. Our goal is to investigate the TLR signaling cascade as a therapeutic target for AD. Our hypothesis is that altering different steps of the TLR signaling pathway will lead to novel strategies to treat AD. Ibudilast (IBU), a

phosphodiesterase (PDE) inhibitor, has already shown promise in treating neurodegenerative conditions such as multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS). Our *in-vitro* studies with the human HMC3 microglial cell line showed that IBU promotes the expression of the anti-inflammatory cytokine IL-13. Our computational studies predicted and subsequently experimentally validated that IBU binds interleukin-1 receptor associated kinase (IRAK1) that plays a key role in TLR signaling. We are thus focusing our studies on the effects of IBU on IRAK1 and TNF receptor-associated factor-6 (TRAF6), as potential mediators of IBU-function. We tested the effects of oral administration of IBU on a transgenic rat model of AD (Tg-AD) and age-matched wild type (WT) controls. The Tg-AD rats express human mutant "Swedish" amyloid-precursor protein (APPsw) and Δ exon 9 presenilin-1 (PS1ΔE9). Tg-AD rats develop cerebral cortical and hippocampal Aβ plaques, neurofibrillary tangles, gliosis, and neuronal loss, as well as cognitive impairment, in an age-dependent progressive manner. Our studies include comparisons among sexes, phenotypes, and two ages: 4 months (pre-pathology) and 11 months, when AD pathology is detected. Using western blotting and immunohistochemical analyses, we are assessing changes in IRAK1 and TRAF6 expression, Tau and Aβ pathology, and gliosis in the rat hippocampi. To evaluate learning ability and memory retention, we used the active place avoidance test (PAT). Our PAT data shows that IBU-treated Tg-AD rats have improved 24-hour memory retention compared to untreated Tg-AD rats, validated by t-test analysis ($p < 0.05$), while no differences in learning were observed across groups during training day. Our results suggest that IBU is a promising drug candidate for AD and give us new mechanistic insights for the designing of new therapeutic strategies to treat AD pathology.

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Poster

212. Alzheimer's Disease and Therapeutic Strategies I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 212.06/E6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: SAF2015-63935R
RTI2018-095793-B-I00
S2017/BMD-3827

Title: Contribution of NOX4 to Tau pathology

Authors: ***P. TRIGO-ALONSO**[1], E. LUENGO[1], C. FERNÁNDEZ-MENDÍVIL[1], I. BUENDIA[3], E. DEL SASTRE-LÓPEZ[1], S. LANTIGUA[2], A. I. CASAS[4], H. H. H. W. SCHMIDT[4], M. G. LOPEZ[5];

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Abstract: Alzheimer's disease (AD) is the most common form of dementia in the elderly characterized by a progressive loss of memory function. Apart from hyperphosphorylation of tau and β-amyloid aggregates, neuroinflammation and oxidative stress are gaining importance in the progression and initiation of AD pathology; NADPH oxidase enzymes (NOXs) play a key role in the latter processes. Furthermore, NOXs seem to participate in perturbations related to cognitive function, and many reports have proposed that NOX may be involved in AD pathogenesis. Although the implication of NOX has been well established in β-amyloid related *in vivo* models, little or nothing is known about the specific contribution to tau pathology, the main driver of neuron toxicity and brain atrophy in AD. In this study we aim to determine the implication of NOX-4 isoform in the progression of tau pathology. For this purpose, NOX-$4^{+/+}$ and NOX-$4^{-/-}$ primary neuronal cultures were treated with adeno-associated viral particles (AAV) containing the P301L human tau and GFP as a control driven by the specific neuronal promoter synapsin-1 (AAV-hTau/GFP). In primary neurons, the suppression of NOX-4 decreased tau hyperphosphorylation measured with AT8 antibody preventing the loss of spine density secondary to AAV-hTau infection. In addition, 4-months old C57/BL6 NOX-$4^{+/+}$ and NOX-$4^{-/-}$ mice were injected with AAV-hTau or AAV-GFP. 7 days after the intracerebroventricular (i.c.v) injection of AAV-hTau, NOX-$4^{+/+}$ mice did not develop cognitive decline, but oxidative stress, inflammation and autophagy impairment were present at this stage. Nevertheless, 28 days post injection, in addition to the alterations mentioned above, AAV-hTau injected NOX-$4^{+/+}$ mice manifest severe cognitive deficit as shown in the long-term potentiation (LTP) *in vivo* experiments, novel object recognition test (NOR), object localization test (OLT), and T-maze. Interestingly, AAV-hTau injected NOX-$4^{+/+}$ showed a selective increase in NOX-4 mRNA, among other isoforms. When AAV-hTau was injected to NOX-$4^{-/-}$ mice, we found decreased accumulation of hyperphosphorylated tau protein, reduction of neuroinflammatory markers, improvement of the autophagic flux, and most importantly, cognitive decline was halted. Therefore, we propose NOX-4 inhibition as a potential new therapeutic target for the treatment of tauopathies such as AD.

Disclosures: P. Trigo-Alonso: None. **E. Luengo:** None. **C. Fernández-Mendívil:** None. **I. Buendia:** None. **E. Del Sastre-López:** None. **S. Lantigua:** None. **A.I. Casas:** None. **H.H.H.W. Schmidt:** None. **M.G. Lopez:** None.

Poster

212. Alzheimer's Disease and Therapeutic Strategies I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 212.07/E7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH SB1: 5R44 AG051302-03

Title: The effects of a novel anti-inflammatory on behavioral tests of cognitive and anxiety in a mouse model of Alzheimer's disease

Authors: *R. W. BROWN[1], H. E. RAUHUFF[1], W. GILL[1], H. W. SHELTON[1], C. W. KERNS[1], S. P. GABBITA[2];
[1]Dept. of Biomed. Sci., East Tennessee State Univ., Johnson City, TN; [2]P2D Bioscience, Inc., Cincinnati, OH

Abstract: Alzheimer's Disease (AD) is a progressive neurodegenerative disease that results in severe cognitive impairment and eventually is fatal. In addition to memory loss, AD patients also present with psychosis and emotional psychological symptoms. In recent years, there has been a research focus on novel treatments for AD, because current medications have not been particularly effective. In the current study, we analyzed whether PD340, a novel anti-inflammatory which inhibits the pro-inflammatory cytokine tumor necrosis factor-alpha (TNFa) would be effective to alleviate behavioral impairments in the 3xTg mouse model of AD. The 3xTg model is unique in comparison to previous rodent models of AD because it expresses three dementia-related transgenes and demonstrates a clear age-dependent onset of AD pathology. Mice were bred in our animal colony. Beginning at four months of age, a specialized diet was presented to the animals that contained 0, 3, 10, or 25 mg/kg of PD340. At approximately 6 months of age, which is before any neuropathology presents in this model, animals were behaviorally tested on three different tasks: the Barnes maze, prepulse inhibition (PPI) and the elevated T-maze. Both males and females were tested. Results from Barnes maze testing revealed that females, but not males, administered 25 mg/kg of PD340 demonstrated a significant improvement in spatial memory. Regarding PPI, there were no sex differences, but groups receiving the 3 or 25 mg/kg dose of PD340 demonstrated significantly improved performance dependent upon the auditory decibel level of the stimulus presented over animals administered a control diet not containing PD340. On the elevated T-maze, there were no significant group differences, demonstrating anxiety is not present at 6 months of age in this model. Employing a repeated measures design, behavioral tests in the same mice will continue to be performed at 12 and 15 months of age to acquire longitudinal data over time. Our data suggests orally administered PD340 is effective in alleviating behavioral deficits related to cognitive impairment in a mouse model of AD. Future work will analyze neuropathology in the hippocampus and prefrontal cortex, two brain areas that degenerate in AD and are important in cognitive function.

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Poster

212. Alzheimer's Disease and Therapeutic Strategies I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 212.08/E8

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Alzheimer's preclinical efficacy database (AlzPED): A new data resource for improving the rigor, reproducibility, transparency and translation of drug efficacy in animal models of Alzheimer's disease

Authors: *L. M. REFOLO, S. CHAKROBORTY, Z. MARTIN, C. SHEFFIELD, S. S. PETANCESKA;
Natl. Inst. on Aging, Bethesda, MD

Abstract: A major challenge to the successful development of therapies for Alzheimer's disease (AD) is the poor translation of preclinical efficacy from animal models to the clinic. Several key factors have been identified as contributors to the unsuccessful translation of therapeutic efficacy. These include: failure of the models to fully recapitulate human AD, poor rigor study design and data analysis, insufficient attention given to using a standard set of "best practices", failure to match outcome measures used in preclinical animal studies and clinical studies, poor reproducibility of published data and publication bias in favor of reporting positive findings. To ameliorate some of the key factors contributing to the preclinical to clinical development gap in AD, the National Institute on Aging (NIA) and the National Institutes of Health (NIH) Library have created a publicly available data repository: AlzPED**.** AlzPED is designed as a web-based knowledge portal for housing, sharing and mining of preclinical efficacy data. The data are submitted to AlzPED through a curator and gleaned from multiple sources. Each study is carefully curated by two experts for data on authors, AD animal models, therapeutic targets and agents, outcomes and most importantly the rigor of the study, prior to publication in the database. AlzPED currently houses curated summaries from 720 preclinical efficacy studies including 149 animal model descriptors, information on 145 therapeutic targets and 643 therapeutic agents, principal findings, and information related to funding sources and financial conflict of interest, and reports on the rigor of each study by summarizing 24 critical elements of experimental design. Analysis of studies curated to AlzPED demonstrates a serious deficiency in reporting critical elements of design and methodology like power/sample size calculation, blinding for treatment and outcomes, randomization, balancing for sex, inclusion/exclusion criteria, resulting in increased susceptibility to misinterpretation and decreased scientific rigor, reproducibility and translational value. AlzPED will provide the AD research community with a facile way to survey existing preclinical therapy development efforts and raise their awareness about the elements of rigorous study design and requirements for transparent reporting. AlzPED will also serve as a platform for reporting unpublished negative findings in order to mitigate the publication bias that

favors the reporting of positive findings. Finally, this new data resource can be used by funding agencies as a tool for enforcement of requirements for transparent reporting and rigorous study design.

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Poster

212. Alzheimer's Disease and Therapeutic Strategies I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 212.09/E9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R21 AG053856-01
Scully Initiative Fund
Jean Perkins Foundation

Title: Small molecule co-activation of TrkB/TrkC neurotrophin receptors prevents cholinergic neuron atrophy in an Alzheimer’s disease mouse model at an advanced pathological stage

Authors: *S. GONZALEZ[1], D. A. SIMMONS[1], T. MCHUGH[1], T. YANG[1], S. M. MASSA[2], F. M. LONGO[1];
[1]Neurol. and Neurolog. Sci., Stanford Univ., Stanford, CA; [2]Dept. of Neurol., SFVAMC & UCSF, San Francisco, CA

Abstract: Degeneration of basal forebrain cholinergic neurons (BFCNs) occurs early in the pathological events leading to memory impairment in Alzheimer’s disease (AD). Aberrant neurotrophin signaling via Trks and the p75 neurotrophin receptor ($p75^{NTR}$) contributes importantly to BFCN dystrophy. Our previous findings identified a small molecule ligand, LM22B-10, that increased cell survival and accelerated neurite outgrowth *in vitro,* and activated TrkB and TrkC signaling in hippocampus of aged mice when delivered intraperitoneally, but showed poor oral bioavailability (Yang et al., 2016). Most AD patients begin treatment at an advanced pathological stage, thus treatment effects in mid- to late-stage pathology may have particular clinical relevance. Therefore, using an orally bioavailable LM22B-10 derivative, PTX-BD10-2, we examined whether co-activation of TrkB/TrkC could prevent and/or reverse BFCN atrophy with treatment beginning at mid-stages of pathology in AD mouse models . PTX-BD10-2 (50 mg/kg) or vehicle (veh; cyclodextran) was administered by oral gavage once daily, 5 days/week, to male Thy-1 $hAPP^{Lond/Swe}$ ($APP^{L/S}$) and wild-type (WT) mice for 4.5 months beginning at age 7.5-9 months, when BFCN atrophy and amyloid deposition is well established. BFCNs in the ventral diagonal band of Broca (VDB) were significantly smaller in veh-treated $APP^{L/S}$ mice at 12-13.5 months of age compared with those in WT mice. BFCNs of $APP^{L/S}$-veh

mice had fewer neurites that occupied a smaller area of the VDB than those in WTs. Moreover, cholinergic neurite branches were atrophied as evidenced by reduced numbers of nodes and ends. PTX-BD10-2 significantly ameliorated cholinergic somal shrinkage and neuritic dystrophy (area, number, and branching) in $APP^{L/S}$ mice. These findings suggest that PTX-BD10-2 can prevent and/or reverse BFCN atrophy in AD mice with advanced pathology. Thus, targeting TrkB and TrkC may represent a promising approach to reducing AD-related degenerative processes that have progressed beyond early stages.

Disclosures: **S. Gonzalez:** None. **D.A. Simmons:** None. **T. McHugh:** None. **T. Yang:** None. **S.M. Massa:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PharmatrophiX. **F.M. Longo:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PharmatrophiX.

Poster

212. Alzheimer's Disease and Therapeutic Strategies I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 212.10/E10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NNSFC 81773717, 81441111, and 81601229

Title: The phosphodiesterase-4 inhibitor roflumilast reverses cognition deficits via cAMP signaling-mediated neuroprotection in 3xTg-AD mice

Authors: *H.-T. ZHANG[1], H. WANG[2], H.-R. FU[2], F.-F. ZHANG[2], Z.-H. XIA[2], Y. CHEN[2], W.-Z. WANG[2], X.-D. WANG[2], L. WANG[2], W. CHEN[2], X.-Y. XU[2], Y.-F. GAO[2], Y. XU[3], J.-G. ZHANG[2];
[1]Departments of Behavioral Med. & Psychiatry, Physiol. & Pharmacology, and Neuroscience, RNI, West Virginia Univ. Hlth. Sci. Ctr., Morgantown, WV; [2]Inst. of Pharmacology, Shandong First Med. Univ. & Shandong Acad. of Med. Sci., Tai'an, Shandong, China; [3]Dept. of Pharmaceut. Sci., Sch. of Pharm. and Pharmaceut. Sciences, Univ. at Buffalo, SUNY, Buffalo, NY

Abstract: Alzheimer's disease (AD) is characterized histopathologically by intraneuronal neurofibrillary tangles, extracellular deposits of β-amyloid as cores of neuritic plaques, and brain neurodegeneration. Phosphodiesterase-4 (PDE4), an enzyme that catalyzes the hydrolysis of cyclic AMP (cAMP), has been considered as a promising target for treatment of memory loss in AD. Here we examined the effects of roflumilast, the second generation of PDE4 inhibitors and an approved drug for treatment of chronic obstructive pulmonary disease (COPD) in humans, on memory loss in 3×Tg-AD mice, a widely used model of AD. 3×Tg-AD mice contain three

mutations (APP Swedish, MAPT P301L, and PSEN1 M146V) in the brain and develop Aβ plaques and tau pathology. Their superior is incomparable in imitating human AD. In the Morris water-maze (MWM) test, the 10-month AD mice displayed significantly longer escape latency during acquisition training and fewer entries into the target quadrant during the probe trial, compared to the age-matched wild-type controls; these were reversed by roflumilast at 1 and 5 mg/kg. Roflumilast also reduced the loss of neurons and neurocyte apoptosis and reversed the decreased ratio of Bcl-2/Bax and the increased expression of PDE4A , PDE4B, and PDE4D in the cerebral cortex and hippocampus of AD mice. Finally, roflumilast reversed the decreases in levels of cAMP, phosphorylated cAMP response element-binding protein (CREB), and brain derived neurotrophic factor (BDNF) in AD mice.These results suggest that roflumilast improves learning and memory in 3×Tg-AD mice, likely via cAMP-CREB signaling-mediated neuroprotection. Therefore, roflumilast can be a therapeutic agent for AD.

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Poster

212. Alzheimer's Disease and Therapeutic Strategies I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 212.11/E11

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Optimization of tissue-selective ABCA1 agonists as an effective and tolerated therapy for Alzheimer’s disease

Authors: *M. BEN-AISSA[1], C. T. LEWANDOWSKI[1], K. M. RATIA[1], B. KARUMUDI[1], M. LADU[2], G. THATCHER[1];
[1]Univ. of Illinois at Chicago (UIC), Chicago, IL; [2]Anat. and Cell Biol., Univ. of Illinois, Chicago, IL

Abstract: Background: Alzheimer’s disease (AD) and related dementia is a multifactorial disease, presenting a challenge to drug discovery. The greatest known risk factor is the *APOE*4-allele with 50% and 90% risk of developing AD for respectively heterozygotes and homozygotes carriers. In the brain, ABCA1 initiates the assembly of Apolipoprotein E (ApoE) containing lipoprotein-particles, which transport lipids throughout the CNS. ApoE4 particles are less stable and poorly lipidated, contributing to loss of function in AD, which can be mitigated by ABCA1 overexpression. Both amyloid-β (Aβ) related- and non-Aβ mechanisms, contribute to the progression of AD, in *APOE4* carriers. Whereas therapeutic approaches solely targeting Aβ, have failed in the clinic, there remains a lack of therapeutic strategies that incorporate *APOE4*. Our objective is to develop a tissue-selective ABCA1 agonist, thereby restoring apoE function

and cholesterol mobilization; in parallel, addressing metabolic dysfunction and other contributors to AD, commonly referenced as Type-3 diabetes (T3D). **Methods:** Therefore, we have taken an innovative functional approach to restore the function of ApoE by screening for compunds that elevate ABCA1 in astrocytes and counter-screen against SREBP1c activation in hepatocytes to mitigate against a detrimental lipogenic genes activation. Selected hits with neutral lipogenic actions were further profiled based upon multifactorial properties for cholesterol metabolism; anti-inflammatory and insulin-sensitizing effects. Toxicodynamic and pharmacodynamics (TD/PD) studies were performed in WT and high-fat diet (HFD) fed mice. Therapeutic potential of ABCA1 inducers and proof of concept study was performed in EFAD murine model of AD that combines five FAD mutations with transgenic replacement of mouse *APOE* alleles with it human homologs. **Results:** From initial 25,000-compounds, 5 Chemotypes were selected for SAR and probed in primary astrocytes for regulation of cholesterol efflux and genes associated with neuroinflammation, energy utilization, insulin sensitivity, and cholesterol metabolism for hit-to-lead optimization. TD/PD data and target-engagement validated the ability of ABCA1 inducers to engage targets *in-vivo* predicted from *in vitro* assays for liver steatosis, plasma and brain biomarkers for multiple mechanisms of action contributing to AD-like behavior and pathology. **Conclusions:** A Preclinical Proof-Of-Concept was established. We propose that this functional approach has potential to impact multiple factors that contribute to AD, both associated with and not directly associated with Aβ.

Disclosures: M. Ben-Aissa: None. **C.T. Lewandowski:** None. **K.M. Ratia:** None. **B. Karumudi:** None. **M. LaDu:** None. **G. Thatcher:** None.

Poster

212. Alzheimer's Disease and Therapeutic Strategies I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 212.12/E12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG043415
Alzheimer’s Drug Discovery Foundation 20160904
Alzheimer Association PTC-18-546300
Alzheimer’s Drug Discovery Foundation 2013554
NIH Grant AG062095

Title: A selective and brain penetrant p38alphaMAPK inhibitor candidate for neurologic and neuropsychiatric disorders that attenuates neuroinflammation and cognitive dysfunction

Authors: M. WINDISCH[1], J. PELLETIER[1], S. M. ROY[2], D. M. WATTERSON[2], *O. ARANCIO[3];

[1]Neurokine Therapeut., New York, NY; [2]Northwestern Univ., Chicago, IL; [3]Columbia Univ., New York, NY

Abstract: Rationale: There is an urgent need to improve, diversify and reinvigorate the Alzheimer's disease (AD) drug development pipeline by accelerating the characterization and experimental validation of non-amyloid therapeutic targets and rapidly integrating qualified therapeutic candidates into human trials to test therapeutic hypotheses. A preponderance of preclinical data and clinical observations established p38αMAPK as a brain drug discovery target involved in neuroinflammatory responses and synaptic dysfunction in multiple degenerative and neuropsychiatric brain disorders. The accumulating body of evidence provides a biological and pharmacological rationale for pursuit of more selective p38αMAPK inhibitor drug candidates with good brain exposure and a therapeutic index that minimizes dose-related adverse pharmacology barriers.
Objective: Develop, for future clinical trials, highly selective small molecule, p38αMAPK inhibitors with good brain exposure that are efficacious in diverse animal models of neurologic disorders and are shown to be safe in FDA guided preclinical development.
Methods & Approach: A crystallography and pharmacoinformatic approach to fragment expansion enabled the discovery of an efficacious hit. The integration of secondary pharmacology screens to medicinal chemistry refinement delivered novel compounds with improved selectivity, appropriate pharmacodynamics and efficacy. FDA guidance on safety considerations and advanced pharmacology screens drove optimization that delivered the qualified therapeutic candidate, MW01-18-150SRM (MW150). MW150 has efficacy in multiple animal models in which synaptic dysfunction and neuroinflammation are contributors to pathophysiology progression.
Conclusions: MW150 is a safe and effective therapeutic candidate that is orally bioavailable, has good brain exposure, and possesses an acceptable therapeutic index that qualified it for human clinical trials.

Disclosures: M. Windisch: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Founder Neurokine Therapeutics. **J. Pelletier:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurokine Therapeutics. **S.M. Roy:** None. **D.M. Watterson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Founding Member of Neurokine Therapeutics. **O. Arancio:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Founder of Neurokine Therapeutics.

Poster

212. Alzheimer's Disease and Therapeutic Strategies I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 212.13/E13

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Funding from Agricultural Research Development Agency, Thailand

Title: Three types of fruit extract of Aegle marmelos reduced amyloid-beta toxicity in a transgenic *Caenorhabditis elegans*

Authors: *R. KEOWKASE, I. KETKAEW, L. NIRANATHMATEEKUL, W. SITTHITHAWORN;
Srinakharinwirot Univ., Nakornayok, Thailand

Abstract: Alzheimer’s disease (AD), a progressive neurodegenerative disorder, is the most common form of dementia found in elderly. The accumulation of amyloid-β (Aβ) is believed to be a major cause of AD. The treatment is limited to a few FDA approved drugs for AD which only temporarily improve symptoms but not cure the disease. Our previous study reported that *Aegle marmelos* (*A. marmelos*), called in Thai as Ma-Toom-Bann, delayed Aβ-induced paralysis in a transgenic *Caenorhabditis elegans* (*C. elegans*) strain CL4176. The objectives of this study were to investigate the effect of other types of *A. marmelos* known as Ma-Toom-Khai and Ma-Toom-Nim against toxicity of Aβ using paralysis and RNA interference (RNAi) assays. We also measured the expression levels of genes involved in insulin/IGF-1 signaling pathway by using Real-Time Polymerase Chain Reaction (RT-PCR). The results demonstrated that three types of *A. marmelos* fruit extract protected against Aβ toxicity because they significantly delayed Aβ-induced paralysis in *C. elegans*. We found that Ma-Toom-Khai and Ma-Toom-Nim failed to delay paralysis when the expression of *daf-16* was knocked down by RNAi. In addition, both types of *A. marmelos* increased the expression level of *daf-16*. We concluded that three types of fruit extract of *A. marmelos* reduced Aβ toxicity, in part, through *daf-16*.

Disclosures: R. Keowkase: None. **I. Ketkaew:** None. **L. Niranathmateekul:** None. **W. Sitthithaworn:** None.

Poster

212. Alzheimer's Disease and Therapeutic Strategies I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 212.14/E14

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: HMRF, Project No: 14151281
MRP-092-17X

Title: Lycium barbarum extracts preserve retinal function by rescuing synaptic loss in 3xTg mouse model of Alzheimer's disease

Authors: *J. LIU, G. XIONG, Y. LIN, R.-C. CHANG, K. SO, K. CHIU;
The Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract: Alzheimer's disease (AD) is a chronic neurodegenerative disease. Ocular abnormalities in patients with AD have been reported, including retinal ganglion cells (RGCs) loss, axonal degeneration in the optic nerve, Amyloid β plaque pathology. The *Lycium barbarum* extracts (LBE) have been demonstrated to be neuroprotective in various animal models of neurodegeneration. In the current study, we aim to investigate the effect of oral feeding LBE on structural and functional changes of retina in the 3xTg AD mouse model. Methods: 6 months old 3xTg-AD and C57/6J mice were treated by gavage with low (200mg/kg) or high (2g/kg) doses of LB hydrophilic extract daily for 3 months, while water treatment was used as a placebo control. Retinal function was evaluated by electroretinogram (ERG) at one month, two months and three months of gavage with a scotopic flash intensity of 3.0 cd.s/m^2 and photopic flash intensity of 22.76 cd.s/m^2. Amplitude and latency of ERG signals were filtered and analyzed by Axon pCLAMP. Mice were sacrificed after 3 months of treatment, and the eyes were harvested. Morphological changes of the retina were analyzed in cross-sections. Immunohistochemical staining (IHC) was performed with various primary antibodies including anti-Brn3a, anti-synaptophysin, and anti-PSD95. Results: ERG results showed that the scotopic retinal response of 3xTg-AD mice started to decline during 4-6 month of age, followed by a rapid decrease during 6-8 month. In this study, one month high doses LBE gavage group showed a significant increase of b-wave in scotopic ERG. The same tendency of b-wave preservation was observed at two months high doses LBE feeding without statistical significance and vanished at three months (9 months of age). In 9 months old AD mice, there were no significant differences in inner nuclear layer thickness, outer nuclear layer thickness, and RGC numbers compared to age-matched C57 mice, and no changes were observed after LBE treatment. However, presynaptic density in Inner plexiform layer but not outer plexiform layer was significantly increased in the LBE high dose treated AD mice, suggesting the presynaptic structure of retina was preserved. Conclusions: Our results indicated that at early- and mid-stage of AD, presynaptic changes in

IPL contributed the most to b-wave decline and LBE gavage could preserve the retinal functional deterioration in 3xTg AD mice by preventing the synapse loss in IPL. The protective effect topped at one month gavage in ERG test and still detectable till the end of three-month-treatment by IHC. This study suggested that LB has the potential to be used as a neuroprotectant in AD-related vision loss.

Disclosures: J. Liu: None. **G. Xiong:** None. **Y. Lin:** None. **R. Chang:** None. **K. So:** None. **K. Chiu:** None.

Poster

212. Alzheimer's Disease and Therapeutic Strategies I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 212.15/E15

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Non clinical profiling of SUVN-A6077, a fast follow up nonchemotype backup of Phase-2a clinical candidate Masupirdine (SUVN-502) in the symptomatic treatment of AD dementia

Authors: R. BADANGE, N. MUDDANA, R. PALACHARLA, R. MEDAPATI, R. SUBRAMANIAN, *J. B. THENTU, V. BENADE, J. TADIPARTHI, N. GANUGA, R. NIROGI;
Suven Life Sci. Ltd., Hyderabad, India

Abstract: Masupirdine (SUVN-502) is a pure 5-HT6 antagonist with 1200 fold selectivity over 5-HT2A receptor. Phase-2a POC study of Masupirdine as triple therapy (Masupirdine added to background treatment with donepezil and memantine) is nearing to completion as a novel approach in the symptomatic treatment of AD dementia. SUVN-A6077 is identified as a fast follow up nonchemotype back for Masupirdine. SUVN-A6077, like Masupirdine is a potent 5-HT6 antagonist (Kb= 0.04 nM) with excellent selectivity over 5-HT2A receptor (Ki = 130 nM). SUVN-A6077 has excellent bioavailability and brain penetration in rats. SUVN-A6077 reversed the time induced memory deficit in novel object recognition task and in fear conditioning model. Following oral administration in combination with donepezil and donepezil + memantine, SUVN-A6077 produced significant increase in acetylcholine levels in rats. Preliminary safety assessment in rats indicates no concerns for its further development. These preclinical data suggest that SUVN-A6077 has therapeutic potential for symptomatic treatment of cognitive deficits, as a fast follow up nonchemotype backup for Masupirdine.

Disclosures: R. Badange: A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **N. Muddana:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **R. Palacharla:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **R. Medapati:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **R. Subramanian:** A.

Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **J.B. Thentu:** A.
Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **V. Benade:** A.
Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **J. Tadiparthi:** A.
Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **N. Ganuga:** A.
Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **R. Nirogi:** A.
Employment/Salary (full or part-time):; Suven Life Sciences Ltd..

Poster

212. Alzheimer's Disease and Therapeutic Strategies I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 212.16/E16

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: DFS-2012

Title: Cadmium-induced oxidative stress and consequent memory loss can be resorted by dietary supplementation of nuts

Authors: *Z. BATOOL[1,2], F. AGHA[4], S. HAIDER[3];
[2]ICCBS, PCMD, [3]Dept. of Biochem., [1]Univ. of Karachi, Karachi, Pakistan; [4]Liaquat Natl. Med. Col., Karachi, Pakistan

Abstract: Exposure to cadmium has become unavoidable and major health concern because of its long biological half-life and its occupational use. Sources of cadmium exposure include consumption of contaminated food and water, and cigarette smoke. Environmental exposure to heavy metal is associated with the deficiency of essential nutrients. Cadmium and other heavy metals interrupt the normal functioning of minerals involved in various metabolic reactions. Oxidative stress is considered as one of the major factors of metal toxicity. Brain is highly sensitive organ due to high metabolic rate and oxygen consumption. Cadmium easily crosses the blood brain barrier and disrupts the normal functioning of brain. Supplementation of indispensable nutrients is suggested to provide protection against the toxicity of heavy metal. Nuts including almond and walnut are highly nutritious foods rich in unsaturated fatty acids, fiber, vitamins, minerals and some bioactive substances, such as phenolic antioxidants and phytosterols. In this study we tested the potential of almond and walnut to ameliorate cadmium-induced oxidative stress in rats. Memory was monitored to analyze the brain function. Study was conducted in two set of experiment with n = 24 for each. Rats were randomly divided into control, almond/walnut, cadmium and alm+cad/wal+cad groups. Rats were orally given cadmium at the dose of 50 mg/kg/week. Whereas almonds and walnuts were administered at the dose of 400 mg/kg/day in the form of suspension. Treatment continued for four weeks followed by memory assessment by Morris water maze (MWM), elevated plus maze (EPM) and novel object recognition (NOR) task. After behavioral analysis rats were decapitated to collect the

brain samples for the estimation of malondialdehyde (MDA) concentration and antioxidant enzyme activity. Results revealed that exposure to cadmium significantly impaired memory function in rats as evident by low memory retention observed in MWM, EPM and NOR. MDA levels and antioxidant enzyme activity were also altered by the exposure of cadmium indicating significant induction of oxidative stress. However, daily supplementation of almond and walnut significantly attenuated cadmium-induced impaired memory and oxidative stress by reducing MDA levels and normalizing enzyme activity. Present study suggests the beneficial effects of constituents present in nuts including essential minerals and antioxidant polyphenols against cadmium-induced toxicity. Regular consumption of nuts can therefore be recommended to combat the unavoidable exposure of cadmium toxicity in daily routine life.

Disclosures: **Z. Batool:** None. **F. Agha:** None. **S. Haider:** None.

Poster

212. Alzheimer's Disease and Therapeutic Strategies I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 212.17/E17

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: KI Research grant

Title: BRICHOS: A potential therapeutic chaperone in Alzheimer’s disease

Authors: *S. MANCHANDA[1], L. G. ACOSTA[2], G. CHEN[2], J. JOHANSSON[2], P. NILSSON[3]; [1]Neurobiology, Care Sci. and Society, Karolinska Institutet, Huddinge, Stockholm, Sweden; [2]Karolinska Institutet, Huddinge, Sweden; [3]Karolinska Institutet, Solna, Stockholm, Sweden

Abstract: Objectives: BRICHOS (Bri2, Chondromodulin, proSP-C) is a chaperone like domain derived from Bri2 (associated with dementia), Chondromodulin (chondrosarcoma), and prosurfactant protein C (interstitial lung amyloid). During biosynthesis it binds to protein regions with high β sheet propensities, thus preventing them from aggregation and amyloid formation. Studies in transgenic model of *Drosphila Melanogaster* have demonstrated that co-expression of Aβ42 and BRICHOS delayed the Aβ aggregation and increased the locomotor activity and life span in flies. We have investigated the therapeutic potential of Bri2 BRICHOS in Alzheimer’s disease (AD) using App^{NL-F} knock-in mice with robust Aβ pathology. These mice are suitable for preclinical AD and also for potential AD therapeutics.
Methods: App^{NL-F}mice (19 months old) harbouring the Swedish (KM670/671NL) and Beyreuther/Iberian (I716F) mutations were injected with PBS (n=10/group) or recombinant human (rh) Bri2 BRICHOS monomer (20mg/kg) intravenously twice a week for a duration of 10 weeks. Post-treatment, mice were evaluated for their anxiety, learning and memory response using a set of different behavioural paradigms (n=8/group) and they will be further evaluated for

their amyloid pathology and neuroinflammation using biochemical assays and protein expression studies.
Results and Conclusions: Elevated plus maze results indicates a tendency of Bri2 BRICHOS treated mice towards reduced anxiety compared to *App*$^{NL-F}$ PBS controls, which instead showed a tendency towards increased anxiety. No change was observed in the alteration score (for working memory) between the two groups post treatment in Y- maze. During contextual fear conditioning, Bri2 BRICHOS treated mice showed higher freezing response (accounted from time spent in freezing) compared to PBS group, which indicates improved aversive learning and memory retention post treatment. These observations suggest a therapeutic potential of rh Bri2 BRICHOS for cognitive recovery in APP KI mice that needs to be corroborated with further molecular studies. In addition, there is need to validate the therapeutic application of Bri2 BRICHOS in other AD models (such as *App*$^{NL-G-F}$) with different paradigms.

Disclosures: S. Manchanda: None. **L.G. Acosta:** None. **G. Chen:** None. **J. Johansson:** None. **P. Nilsson:** None.

Poster

212. Alzheimer's Disease and Therapeutic Strategies I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 212.18/E18

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: TauRx Therapeutics Ltd, Singapore

Title: Differential effects of treatment with LMTM on rivastigmine and memantine induced changes in acoustic startle response

Authors: L. ROBINSON[1], E. DREESEN[1], P. ARMSTRONG[1], C. R. HARRINGTON[1,2], C. M. WISCHIK[1,2], *G. RIEDEL[1];
[1]Sch. of Medicine, Med. Sci. and Nutr., Univ. of Aberdeen, Aberdeen, United Kingdom; [2]TauRx Therapeut., Singapore, Singapore

Abstract: The cholinesterase inhibitor rivastigmine and the NMDA antagonist memantine are frequently utilised in the therapeutic treatment of Alzheimer’s disease and Frontotemporal dementia (FTD). In our previous studies chronic treatment with rivastigmine and memantine were observed to induce increased levels of reactivity/activity to stimuli in L66 mice which have previously been reported to be a useful mouse model for FTD. The objective of the present study was to assess acoustic startle response as a measure of reactivity/activity in the L66 mice following chronic administration with either of the two treatments and determine the combination effect with Leucomethylthioninium (LMTM), a tau aggregation inhibitor. Female NMRI and L66 tau transgenic mice (Charles River, UK), aged 5-6 months, were pre-treated with

rivastigmine (0.5 mg/kg), memantine (20 mg/kg) or vehicle via oral gavage for 4 weeks whilst being continuously monitored for reactivity to stimuli. In week 4 the startle response of the mice was assessed using acoustic startle/PPI chambers (Med Associates). The acoustic startle test consisted of habituation to 70dB white noise followed by random delivery of startle pulses (volumes 80, 90, 100, 110 or 120dB or null stimuli). Startle amplitude was determined and compared for each treatment group as an indicator of reactivity. Following matching for performance, animals were then administered with either memantine or rivastigmine alone or in combination with LMTM (5 or 15 mg/kg) for a further two weeks before startle responses were performed again. Rivastigmine but not memantine induced a significant increase in the startle response of L66 mice an effect which was ameliorated by co-administration with LMTM. These results suggest that rivastigmine and memantine induce different behavioural effects both alone and in combination with LMTM. A combination therapy of rivastigmine with LMTM has potential beneficial effects in tau transgenic mice. However, further studies are required to determine the mechanisms underlying the differential effects observed with rivastigmine and memantine.

Disclosures: L. Robinson: None. **E. Dreesen:** None. **P. Armstrong:** None. **C.R. Harrington:** A. Employment/Salary (full or part-time):; TauRx Therapeutics Ltd, Singapore. **C.M. Wischik:** A. Employment/Salary (full or part-time):; TauRx Therapeutics Ltd, Singapore. **G. Riedel:** None.

Poster

212. Alzheimer's Disease and Therapeutic Strategies I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 212.19/E19

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Inhibition of tau phosphorylation *in vitro* and *in vivo* by GSK3 alpha isoform-selective antagonists

Authors: T. URQUHART, *B. AMARAL, M. CALHOUN, O. GOLONZHKA, S. HE, D. RABAH, S. KOIRALA;
Biogen, Cambridge, MA

Abstract: Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by memory loss and cognitive decline and is the most prevalent form of dementia. While the causes of sporadic AD are unknown, Tau protein is thought to play an important role in disease etiology and progression. In its normal state, Tau binds to and stabilizes microtubules. When Tau becomes hyperphosphorylated in the disease state, it dissociates from microtubules and aggregates into neurofibrillary tangles (NFTs). Loss of normal function of Tau and gain of toxic function due to hyperphosphorylation and NFT formation are both implicated in AD. Glycogen

synthase kinase 3 (GSK3) phosphorylates Tau at multiple sites relevant to disease histopathology. It exists as two highly similar isoforms, GSK3α and GSK3β. Dual inhibition of GSK3α and β reduces tau phosphorylation (pTau) but has been associated with toxicity due to β-Catenin stabilization via the Wnt pathway, leading to tissue hyperplasia. We hypothesized that isoform-selective inhibition or reduction of GSK3 could provide effective p-Tau inhibition while minimizing risk of hyperplasia. Focusing principally on GSK3α, we used novel GSK3α-selective antagonists (~10-50x selective for α over β) and isoform-selective siRNAs/shRNAs in multiple *in vitro* and *in vivo* systems to assess effects on p-Tau and tissue hyperplasia. First, we established selectivity of novel compounds using a cell system expressing human P301S Tau and overexpressing GSK3α or β. Second, in primary rat neurons, we confirmed robust pTau reduction with GSK3α-selective compounds. In complementary studies, similar effects were observed with GSK3α shRNAs. Third, to assess effects of GSK3α-selective antagonists *in vivo*, we utilized postnatal day 10 rats, which have physiologically elevated levels of phosphorylated Tau in the brain as well as low PGP transporter expression, facilitating brain exposure of our compounds. We observed substantial p-Tau inhibition *in vivo* that correlated with drug levels, suggesting that antagonists preferentially inhibiting GSK3α could be effective in reducing Tau pathology. In order to assess effects of isoform selective GSK3 reduction on peripheral tissue hyperplasia, we injected mice i.v. with GSK3α siRNA in lipid nanoparticles. We observed that >70% reduction of GSK3α for 4 weeks in the liver caused no obvious effects on cell proliferation or β-catenin levels. In summary, we determined that novel inhibitors that preferentially inhibit GSK3α retain the ability to reduce tau phosphorylation *in vitro* and *in vivo* while mitigating potential toxicity due to tissue hyperplasia.

Disclosures: **T. Urquhart:** A. Employment/Salary (full or part-time):; Biogen. **B. Amaral:** A. Employment/Salary (full or part-time):; Biogen, Inc. **M. Calhoun:** A. Employment/Salary (full or part-time):; Biogen. **O. Golonzhka:** A. Employment/Salary (full or part-time):; Biogen. **S. He:** A. Employment/Salary (full or part-time):; Biogen. **D. Rabah:** A. Employment/Salary (full or part-time):; Biogen. **S. Koirala:** A. Employment/Salary (full or part-time):; Biogen.

Poster

212. Alzheimer's Disease and Therapeutic Strategies I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 212.20/E20

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: NSX-0527, an M1/M4 selective muscarinic agonist for schizophrenia and Alzheimer's disease

Authors: *J. D. BECK, J. OCKULY, S. HANSON, M. HENDRICKSON;
Neurosolis, Inc, Madison, WI

Abstract: Muscarinic agonists have long held promise for CNS disorders including schizophrenia and Alzheimer's disease (AD). The M1/M4-preferring agonist xanomeline showed efficacy in both AD patients and schizophrenia patients. Interestingly, in addition to cognitive benefits, the compound reduced vocal outbursts, suspiciousness, delusions, agitation, and hallucinations. Ultimately, xanomeline was abandoned due to intolerable side effects. NSX-0527 is an M1/M4 preferring, orthosteric, muscarinic agonist showing good bioavailability (~75%), brain penetration (~60%), and excellent metabolic stability. NSX-0527 showed efficacy in rodents in behavioral antipsychotic assays including: reversal of apomorphine-induced climbing, reversal of MK-801- and amphetamine-induced hyperlocomotion, and inhibition of conditioned avoidance response. NSX-0527 compared favorably to xanomeline as well as olanzapine without producing the classic antipsychotic side effect of hyperprolactinemia. In an effort to improve tolerability for high oral doses of this compounds, we explored combinations of NSX-0527 and an FDA-approved peripheral muscarinic agonist. At very low antagonist/agonist dose ratios, peripheral muscarinic activity was abolished with no discernable effect on central (CNS) activity in a variety of *in vivo* assays. Thus, a fixed-dose combination may allow for high agonist doses to aggressively drive central muscarinic M1 and M4 activation for a therapeutic benefit with minimal side effects.

Disclosures: **J.D. Beck:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroSolis ownership interest. **J. Ockuly:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroSolis ownership interest. **S. Hanson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroSolis ownership interest. **M. Hendrickson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroSolis ownership interest.

Poster

212. Alzheimer's Disease and Therapeutic Strategies I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 212.21/E21

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: AZP2006 a first in class new neuroprotective compound for the treatment of Alzheimer's disease and related neurodegenerative diseases

Authors: *P. VERWAERDE[1], C. ESTRELLA[1], S. BURLET[1], A. HENRIQUES[2], N. CALLIZOT[1];
[1]Alzprotect, Loos, France; [2]Pharmacol., Neuro-Sys, Gardanne, France

Abstract: Alzheimer disease (AD) affects mainly people over the age of 65 years, suffering from different clinical symptoms such as progressive decline in memory, thinking, language, and learning capacity. AD is pathologically characterized by extracellular senile plaques composed of amyloid-β (Aβ) peptides (Aβ plaques) and intracellular neurofibrillary tangles composed of hyperphosphorylated tau. Synapse loss is widespread and pronounced. In AD, the chronic Aβ accumulation causes cerebral neuroinflammation by activating microglia.
AZP2006 is currently in clinical development phase 2a in Progressive Supranuclear Palsy (PSP) patients. We previously showed that AZP2006 was protective in different vivo and vitro models of PSP reducing the hyperphosphorylation of Tau protein, the deposits in animals and the cognitive deficits. We showed that AZP2006 was able to reduce the neuroinflammation and could protect neurons via the release of a growth factor: the progranulin (PRGN).
Here, we investigated the neuroprotective effect in *in vitro* /*vivo* models of AD. In addition, mechanistic studies were conducted to deeply investigate its mode of action.
Using primary culture of cortical/hippocampal neurons injured with in Aβ1-42 oligomers in presence and absence of microglial cells we showed that AZP2006 rescued neurons from the injury reducing the hyperphosphorylation of Tau, protecting synapses and neurite connections.
Using *in vivo* models, we showed that AZP2006 was able to protect but also to restore cognitive impairment, to increase the synapse number, to reduce the microglial inflammation and the hyperphosphorylated Tau protein accumulation.
In addition, mode of action studies showed that AZP2006 displayed a high affinity for the lysosomes and was able to increase the PRGN/Prosaposin complex stability and finally the release of PRGN involved in the neuron survival and neurite outgrowth. We showed that the inhibition of these factors fully abolished the neuroprotective effect and the synaptogenesis induced by AZP2006. In addition, AZP2006 was able to antagonize Toll-like receptor 9 (TLR9) highly involved in the inflammatory process. This dual effect makes AZP2006 a serious candidate for the treatment of neurodegenerative disorders such as AD.

Disclosures: **P. Verwaerde:** A. Employment/Salary (full or part-time):; Alzprotect. **C. Estrella:** A. Employment/Salary (full or part-time):; Alzprotect. **S. Burlet:** A. Employment/Salary (full or part-time):; Alzprotect. **A. Henriques:** None. **N. Callizot:** A. Employment/Salary (full or part-time):; Alzprotect.

Poster

212. Alzheimer's Disease and Therapeutic Strategies I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 212.22/E22

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 1T32AG057468

Title: Small molecule ABCA1 inducers as potential multifunctional therapeutics for Alzheimer's disease

Authors: *C. T. LEWANDOWSKI[1], M. BEN AISSA[2], M. KHAN[1], O. DUBROVSKYI[1], B. KARUMUDI[1], B. LAYDEN[1], M. LADU[3], G. THATCHER[1];
[1]Univ. of Illinois, Chicago, IL; [2]Col. of Pharm. (UIC), Chicago, IL; [3]Anat. and Cell Biol., Univ. of Illinois, Chicago, Chicago, IL

Abstract: There are currently no disease-modifying therapies for Alzheimer's disease (AD). Clinical trials with amyloid-targeting agents have consistently failed, indicating an urgent need to explore novel therapeutic avenues. The connection between AD and type 2 diabetes (T2D), underlined by shared deficits in lipid metabolism, insulin signaling, and inflammation, represents one such avenue. Literature reports show that increased expression of the cholesterol transporter ABCA1 corrects these deficits; elevated ABCA1 should also increase lipidation and function of apoE and therefore provide added benefit to *APOE* ε4carriers. However, transcription factors controlling ABCA1 also promote hepatic triglyceride synthesis (via SREBP1c protein), so non-selective activity leads to fatty liver disease. Our objective is to develop novel, selective small molecule inducers of ABCA1 that possess multifunctional therapeutic effects without impacting peripheral lipogenesis. To that end, we first conducted a luciferase-based high-throughput screen to identify compounds that increased ABCA1, but not SREBP1c, expression. Following validation, structural analogs of a selected hit were synthesized to identify new compounds with enhanced potency. Through multiple synthetic iterations, we developed a lead compound that demonstrated sub-micromolar potency toward ABCA1 induction *in vitro* while maintaining minimal effect on SREBP1c. This optimized molecule was further validated in multiple phenotypic cell-based assays, in which we observed increased cholesterol transport and reduced inflammatory response to LPS stimulation following treatment. Finally, we tested this compound in high-fat diet (HFD) mice, demonstrating diminished weight gain and adipose tissue deposition along with heightened insulin sensitivity in probe- vs. vehicle-treated mice. Notably, probe treatment actually decreased plasma and liver triglycerides in these mice, showing the promise of our strategy to develop small molecules with multifactorial ABCA1-mediated therapeutic activity but minimal adverse effects. Additional work is ongoing to evaluate and optimize pharmacokinetic and metabolic properties, in preparation for long-term studies in AD mouse models to explore probe efficacy at correcting cognitive deficits and AD pathology associated with *APOE4*.

Disclosures: C.T. Lewandowski: None. **M. Ben Aissa:** None. **M. Khan:** None. **O. Dubrovskyi:** None. **B. Karumudi:** None. **B. Layden:** None. **M. LaDu:** None. **G. Thatcher:** None.

Poster

212. Alzheimer's Disease and Therapeutic Strategies I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 212.23/E23

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Drug Discovery Foundation
The Bright Focus Foundation
NIA U01AG05444
The Harrington Foundation

Title: Development of pyridazine-derivatives for the treatment of neurological disorders

Authors: *J. B. FOSTER[1], X. WANG[1], R. LASHLEY[1], F. ZHAO[1], Z. XU[1], K. HODGETTS[2], C.-L. G. LIN[1];
[1]Neurosci., The Ohio State Univ., Columbus, OH; [2]Neurol., Brigham and Women's Univ., Cambridge, MA

Abstract: Glutamate is the primary excitatory neurotransmitter in the forebrain; however, excessive glutamate can cause synaptic damage and neuronal death. Excitatory amino acid transporter 2 (EAAT2) is the principle forebrain glutamate transporter and tightly regulates synaptic glutamate homeostasis maintaining low basal concentrations. Many neurological diseases share a common feature of reduced EAAT2, glutamate dyshomeostasis, and synaptic loss. Therefore, we screened for compounds that increase EAAT2 expression in astrocytic processes via translational activation. From this screen, we identified a pyridazine-based compound-series that normalizes glutamate homeostasis via increased EAAT2 expression. Moreover, using advanced compound derivatives, we have found that pyridazine-derivatives enhance the overall tripartite synapse by concurrently enhancing astrocytic process glutamate uptake function and synaptic bouton function as assessed by increased PSD95 expression and hippocampal long-term potentiation. Recently, we have focused our efforts on two compounds as clinical candidates. Each compound exhibited excellent pharmacokinetics such as high solubility in water (>1 mg/µl), robust oral bioavailability, and remarkable brain penetration (~2:1 brain:plasma ratio; brain cMax > 10 µM at dose of 10 mg/kg PO) and low toxicity. Each compound robustly increased EAAT2 expression as well as PSD95 expression to higher levels than previous compounds. Increased EAAT2 expression was also confirmed in higher order animals (dog) and in human specimen assays (primary astrocytes and post-mortem tissue *ex vivo*). In previously published studies, we have shown that pyridazine-derivatives exhibit efficacy in multiple neurological disease models including Alzheimer’s disease (both amyloid and tau models), epilepsy, and amyotrophic lateral sclerosis. Preliminary data suggest that these compounds may also be efficacious for the treatment Gulf War Illness. Currently, we are testing

efficacy of the clinical candidates in each of these models. Together, the pharmacokinetics, pharmacodynamics and robust efficacy of pyridazine-derivatives in multiple animal models of neurological disease show promise for translation as a therapeutic to human clinical populations.

Disclosures: **J.B. Foster:** None. **X. Wang:** None. **R. Lashley:** None. **F. Zhao:** None. **Z. Xu:** None. **K. Hodgetts:** None. **C.G. Lin:** None.

Poster

212. Alzheimer's Disease and Therapeutic Strategies I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 212.24/E24

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: The Strategic Research Program for Brain Sciences, the Japan Agency for Medical Research and Development (AMED) 18dm0107070h0003

Title: The preventive approach for Alzheimer’s disease based on the novel neprilysin regulator

Authors: *N. WATAMURA, N. KAKIYA, T. SAITO, T. SAIDO;
Ctr. for Brain Science, Proteolytic Neurosci., RIKEN, Wako-shi/Saitama, Japan

Abstract: Alzheimer’s disease (AD) is the most common cause of dementia among the elderly. One of the pathological hallmarks of AD is accumulation of senile plaques composed of amyloid-β peptide (Aβ) in the brain. Neprilysin (NEP), typeII transmembrane endopeptidase, is a potent Aβ-degrading enzyme. Therefore, identification of the mechanism(s) that would induce NEP activation may lead to development of an effective preventive method against AD. Previously, we showed that somatostatin (SST), one of the neuropeptides released from inhibitory neurons, regulates NEP activity. In addition, we have found that somatostatin receptor (SSTR) subtypes 1 and 4 among 5 subtypes redundantly regulate the activation and activity status of NEP. We have thus investigated the molecular mechanism(s), by which SST and SSTR1/4 regulate NEP activity. Recently, we found that the cerebral cortex/hippocampus-basal ganglia communication plays an important role in upregulation NEP *in vivo* upon SST stimulation. We have thus focused our attention on the cell-cell communication mechanisms. Initial proteomic analysis using the culture media from primary neurons treated with SST identified α-endosulfine (ENSA), an endogenous ligand for the potassium channel, as a novel NEP regulator. We then found that genetic deficiency of ENSA decreases Aβ levels presumably due to an increase in NEP activity in the hippocampus via its translocation to the presynaptic membrane from intraneuronal vesicles. Finally, an oral administration of diazoxide, a potassium channel modulator, for 3 months decreased Aβ pathology in cerebral cortex and hippocampus particularly in subiculum and molecular layer of single *App* knock-in mice and improved the memory impairment. This is likely to have been mediated by activation of NEP. These results

indicate that the new mechanisms of NEP activation that we have found may contribute to development of an alternative preventive measure against AD in a preclinical stage.

Disclosures: **N. Watamura:** None. **N. Kakiya:** None. **T. Saito:** None. **T. Saido:** None.

Poster

212. Alzheimer's Disease and Therapeutic Strategies I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 212.25/E25

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Supported by Fondecyt 1180752

Title: Drug repurposing to block multiple steps in the amyloid beta toxicity cascade

Authors: *L. G. AGUAYO, J. GONZALEZ-SANMIGUEL, D. BASCUÑÁN, E. FERNANDEZ-PEREZ, N. RIFFO, C. PETERS, F. BURGOS;
Physiol., Univ. Concepcion, Concepcion, Chile

Abstract: One of the main neurotoxic agents of Alzheimer´s disease (AD) corresponds to diffusible oligomers of the β amyloid peptide (Aβ) that is released to the extracellular compartment associating to the neuronal membrane and forming "pore-like" structures. These membrane perturbations are able to elevate intracellular calcium increasing neurotransmitter release that produces late synaptic failure and death. Current anti-AD therapies are not effective, thus new approaches are needed. While screening for small molecules that associate with the C terminal region of Aβ and are able to interfere with the association and perforation of the neuronal membrane, we detected an FDA approved compound that has interesting properties at blocking multiple toxic steps of Aβ such as aggregation, association, intracellular calcium increase, and synaptotoxicity. We examined a large library of synthetic compounds in order to find small weight molecules that have similar neuroprotective effects, but with higher pharmacological potential, such as brain penetration. An FDA approved compound was found to have *in silico* interaction with Aβ. Subsequently, the compound was tested using an aggregation assay, in vitro Aβ-FAM association, mitochondrial function, and synaptic transmission in hippocampal neurons. The application of Aβ oligomers to hippocampal neurons resulted in an array of effects starting at the cell membrane and leading to toxicity within 48 hrs. We found that the active compound, at micromolar concentrations, was able to antagonize Aβ aggregation over time, reducing Aβ absorbance plateau to 28% of control. It also reduced hippocampal membrane association, a step necessary for early toxicity, by about 50%. In addition, the effects of Aβ on intracellular calcium were antagonized, without causing effects on its own. Finally, using patch clamp recordings and immunocytochemistry, we found that the compound was able to block the synaptotoxicity induced by Aβ in hippocampal neurons increasing postsynaptic currents from 1.7

± 0.9 to 4.2 ± 0.7 pA·ms, and SV2 mean relative levels from 0.7 ± 0.09 to 1.00±0.08. The results show that an FDA approved compound is able to interfere with Aβ-induced toxicity by blocking multiple steps resulting in synaptic protection.

Disclosures: L.G. Aguayo: None. **J. Gonzalez-Sanmiguel:** None. **D. Bascuñán:** None. **E. Fernandez-Perez:** None. **N. Riffo:** None. **C. Peters:** None. **F. Burgos:** None.

Poster

212. Alzheimer's Disease and Therapeutic Strategies I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 212.26/E26

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Genetic editing of human amyloid beta 1-42 into a low-aggregating, low-toxic species via CRISPR/Cas9 technology

Authors: *M. MARCATTI[1], M.-A. MICCI[2], G. TAGLIALATELA[3];
[2]Dept. of Anesthesiol., [3]Neurol., [1]The Univ. of Med. Br. at Galveston, Galveston, TX

Abstract: Alzheimer’s Disease (AD) is a progressive neurodegenerative disorder that causes age-associated dementia. Currently 5.5 million of Americans are affected by AD and that number is expected to triple by 2050. At the present time, it represents the third leading cause of death behind heart disease and cancer. AD neuropathology is characterized by the presence of senile plaques composed of misfolded aggregated amyloid beta (Aβ) and hyperphosphorylated tau which aggregates to form the neurofibrillary tangles. Aβ oligomers represent one of the driving element of AD neurodegeneration and any attempt to stop it may have therapeutic potentials. Here we propose the use of the CRISPR/Cas9 gene editing methodology as a preventive/therapeutic approach to stop the aggregation of Aβ. We selectively edited the human Aβ gene sequence in order to be stably converted in a murine Aβ that differs only for three aminoacids which make it unable to aggregate and trigger the toxicity at the basis of the AD. We edited the Aβ sequence in the CHO-derived 7PA2 cells that express the human amyloid precursor APP and produce highly toxic Aβ oligomers. We performed a target DNA sequencing of the edited 7PA2 (e-7PA2) to validate the proper APP editing and we used the human differentiated neuroblastoma cell line SH-SY5Y to evaluate the off-target CRISPR/Cas9 editing. We compared the presence of Aβ oligomers in the conditioned media of 7PA2 and e-7PA2 cell lines by western blot and ELISA and we evaluated the changes in the induced neuronal toxicity by performing cytotoxicity assays (LDH, MTT, Annexin V) in both cell lines. Moreover, we compared the effect of human and murine Aβ in the synaptic function by using the field recording methodology. Overall our studies aim to demonstrate the possible use of a novel genetic approach to eliminate only the toxic misfolded form of Aβ without affecting its endogenous levels and its physiological function.

Disclosures: **M. Marcatti:** None. **M. Micci:** None. **G. Taglialatela:** None.

Poster

212. Alzheimer's Disease and Therapeutic Strategies I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 212.27/E27

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Klotho impact on Alzheimer's disease pathogenesis through TRPC channel modulation

Authors: *S. CHAKRABORTY[1], S. ACHARYA MALKARAM[2], H. DONG[1];
[1]Northwestern Med. Chicago, Chicago, IL; [2]Dept. of Biol., West Virginia State Univ., Institute, WV

Abstract: Klotho is a single-pass transmembrane anti-aging protein that significantly enhances cognitive function. Recent studies indicate that Klotho -/- mice demonstrate accelerated development of aging-like phenotypes including stunted growth, less bodyweight, thin and atrophic skin and cognitive deficits. Despite Klotho's ability to mitigate AD-related cognitive decline, whether Klotho also affects the neuropathgenesis of AD and the mechanism underlying cognitive deficits are still unknown. Klotho by its β-glucuronidase activity acts as a mediator of transient receptor potential cation TRPV5 channel trafficking. α-klotho protein fragment (αKL-F), administered peripherally, induced cognitive enhancement and neural resilience in mice. γ-secretase-mediated cleavage of pre-Aβ intermediates is the final step in Aβ production and is highly implicated in AD pathogenesis. Interestingly, it was shown that TRPC6 interacts with APP leading to inhibition of its cleavage by γ-secretase and, thus, a reduction in Aβ production. Therefore, we hypothesize that Klotho impact on AD neuropathgenesis through the regulation of the TRPC channels function. We analyzed brain-specific AD and control transcriptome sequence datasets from whole brain, frontal lobe and temporal lobe to determine gene profiles and differential gene expression in AD subjects compared to controls. We focused mainly on Klotho, TRPCs genes considering their role in Alzheimer's disease pathogenesis and progression. We found that the TRPC1, TRPC3, TRPC5, as well TRPC6 were down-regulated >3-fold in the cortex. Additionally, Klotho gene were also found to be down regulated by 3-fold in the same areas.
Our biochemical studies by western blot analysis demonstrated that Klotho and TRPC1, TRPC5 and TRPC6 were significantly downregulated in AD post-mortem brain tissue and in the brains of AD mouse models suggesting that these molecules may be instrumental in AD pathogenesis. In a 13-month old AD knock-in mouse brain (APP^{NL-G-F}), Klotho and TRPC6 co-localize in cortical neuronal membrane, further supporting that Klotho impact on AD may through TRPC6 channels trafficking and influence γ-secretase-mediated cleavage in the AD. To confirm this link, next we will conduct the chromatin immunoprecipitation (ChIP) assay for Klotho-TRPC6 on γ-

secretase gene promoter and overexpression of Klotho, by viral vector-mediated genome editing approach, in the brains of AD mice to improves the entrapment of TRPC channels in the plasma membrane and decrease Aβ production. Our work may open a novel avenue for the therapeutic potential for the treatment of AD.

Disclosures: S. Chakraborty: None. **H. Dong:** None. **S. Acharya Malkaram:** None.

Poster

213. Parkinson's Disease: Molecular Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 213.01/E28

Topic: C.03. Parkinson's Disease

Support: Supported by a grant from Qilu Pharmaceutical Co., Ltd.

Title: Dysregulation of sphingolipid expression and metabolism and its role in the pathogenesis of Parkinson's disease

Authors: *J. S. SCHNEIDER, V. SINGH;
Thomas Jefferson Univ., Philadelphia, PA

Abstract: The precise mechanisms initiating and perpetuating the cellular degeneration in Parkinson's disease (PD) remain unclear. A potentially important pathogenic mechanism contributing to the neurodegeneration in PD is dysfunctional sphingolipid synthesis and metabolism. Gangliosides are glycosphingolipids bearing a ceramide anchor, an oligosaccharide, and one or more sialic acid residues. Gangliosides are enriched in plasma and intracellular membranes and their expression can change in response to external and internal environmental demands. These changes can have positive or negative consequences on cell integrity and survival, depending on the circumstances. In addition to gangliosides, other sphingolipids, such as sphingosine-1-phosphate (S1P) may play important roles in the pathophysiology of PD. We have conducted various studies using human post-mortem tissue to assess alterations in ganglioside biosynthesis and expression in PD. Dopamine (DA) neurons were extracted from normal and PD substantia nigra (SN) using laser capture microdissection followed by RNA extraction, generation of RNA amplicon libraries for use with a custom AmpliSeq panel (focused on glycomics and trophic factors) and Next-Gen sequencing using the Ion Personal Genome Machine (PGM) System. Results suggest that glycolipid dysregulation in SN DA neurons in the PD brain may be more extensive than previously thought and includes altered expression of glycosyltransferases, sialyltransferases, and sialidases, as well altered expression of signaling molecules including neurotrophic factors (ex., GDNF, FGF2) and their receptors (ex., FGFR1, NGFR, FGFR2, GFRA1, GFRA2). Differences in expression were also noted in cells from medial vs. lateral SN, reflecting their differential vulnerability to degeneration in PD. We also

performed in situ hybridization histochemistry and found gene expression of B3GALT4 and ST3GAL2 significantly decreased in residual DA neurons in the SN in PD cases, supporting findings of decreased expression of GM1, GD1a, GD1b, and GT1b in PD SN. We also show for the first time that sphingosine kinase 1 (SPHK1) levels are significantly decreased in the SN in PD, compared to age-matched non-PD controls, suggesting alterations in the sphingosine-1-phosphate (S1P) metabolic pathway in PD. The picture that emerges is one of a complex dysregulation of sphingolipid synthesis and metabolism in PD that may play an important role in the initiation and progression of PD pathology.

Disclosures: J.S. Schneider: None. **V. Singh:** None.

Poster

213. Parkinson's Disease: Molecular Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 213.02/E29

Topic: C.03. Parkinson's Disease

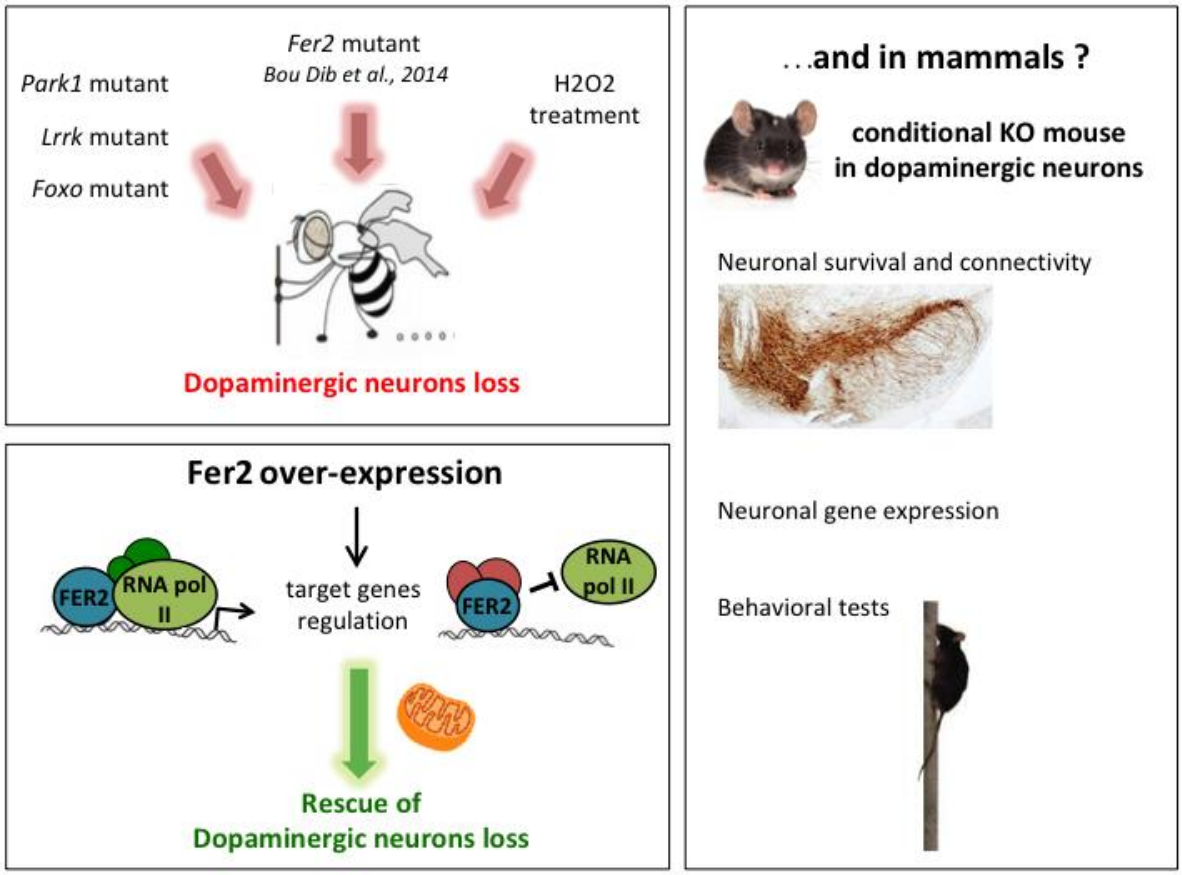
Support: ERC

Title: Investigating neurodegeneration in a novel model of Parkinson's disease: A neuroprotective transcription factor conserved from the fly to mammals

Authors: *F. MIOZZO[1], L. STICKLEY[1], D. TAS[2], N. LONCLE[3], M. DORCIKOVA[1], E. NAGOSHI[1];
[1]Univ. of Geneva, Geneva, Switzerland; [2]Neurimmune Holding AG, Zurich, Switzerland; [3]CHUV Lausanne Univ. Hosp., Lausanne, Switzerland

Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disorder, primarily caused by the specific and progressive loss of dopaminergic (DA) neurons in the *Substantia Nigra*. Despite the discovery of several genes implicated in PD, the scarcity of animal models showing robust and progressive loss of DA neurons has hindered the study of the pathology, and no protective or restorative therapies are currently available.
We have recently established a novel PD model in *Drosophila*, the *Fer2* gene loss-of-function mutant, which recapitulates several hallmarks of PD, notably the specific loss of DA neurons, severe locomotor impairment and mitochondrial defects (Bou Dib et al., PLoS Genetics, 2014). Moreover, *Fer2* over-expression in DA neurons is sufficient to rescue neurodegeneration in a number of genetic PD models and upon oxidative insults, further highlighting *Fer2* importance for protection of DA neurons.
Since *Fer2* is a transcription factor, we hypothesized that it controls a specific set of target genes to exert its beneficial role. By combining Chromatin Immunoprecipitation coupled to sequencing (ChIPseq) and transcriptome analysis (RNAseq), we identified over 30 *bona fide* direct targets,

bound and regulated by *Fer2*. A knock-down/over-expression screening functionally validated several of these genes as novel actors in DA neurons survival. Furthermore, profiling of DA neurons-specific transcriptome revealed *Fer2* regulation of multiple components of the electron transport chain, pointing out a crucial role for mitochondria in *Fer2*-medited neuroprotection. Current investigation of *Fer2* mammalian counterpart, through its conditional inactivation in mouse, is revealing the importance of this gene for DA neurons gene expression and physiology, and for mouse locomotion. Elucidation of the genes and processes governed by *Fer2* across evolution will provide novel insights into the degeneration of DA neurons, and potential new targets for therapeutic interventions.



Disclosures: **F. Miozzo:** None. **L. Stickley:** None. **D. Tas:** None. **N. Loncle:** None. **M. Dorcikova:** None. **E. Nagoshi:** None.

Poster

213. Parkinson's Disease: Molecular Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 213.03/E30

Topic: C.03. Parkinson's Disease

Support: American Parkinson Disease Association Research Grant

Title: Cytisine and estrogen exert synergistic neuroprotection in a 6-OHDA mouse model of Parkinson's disease

Authors: *S. M. ZARATE, G. PANDEY, N. A. SALEM, B. CUDE, S. STOREY, S. CHILUKURI, J. A. GARCIA, T. E. HUNTINGTON, E. BANCROFT, M. HOOK, R. SRINIVASAN;
Neurosci. and Exptl. Therapeut., Texas A&M Hlth. Sci. Ctr., Bryan, TX

Abstract: Parkinson's disease (PD) incidence rates predict a worldwide pandemic that will affect over 12 million people by 2040, which underscores an urgent need for neuroprotective drugs. Unfortunately, most neuroprotective drugs fail clinical trials because PD is caused by a variety of insults that are not recapitulated in any single animal model. We approach this barrier by focusing on hyperactivated endoplasmic reticulum (ER) stress, which is a convergent apoptotic mechanism for multiple PD-related toxicities. We found that in dopaminergic (DA) neurons from 4-week old primary mouse midbrain cultures, 3 week exposure to 200 nM cytisine, a partial agonist of neuronal nicotinic acetylcholine receptors (nAChRs) upregulated Sec24D containing Endoplasmic Reticulum Exit Sites (Sec24D-ERES). Since cytisine-mediated Sec24D-ERES upregulation occurred at a 200 nM concentration, which is incapable of activating surface receptors, we rationalized that this effect was independent of surface nAChR activation and likely occurs via chaperoning of nAChRs out of the ER. We predicted that cytisine-mediated Sec24D-ERES upregulation would generally increase the co-export of proteins out of the ER, thereby reducing protein burden in DA neurons and attenuating ER stress. As predicted, cytisine partially inhibited the ER stress response induced by 48 h exposure to 400 μM 6-hyroxydopamine (6-OHDA). Specifically, cytisine attenuated the activation of two key ER stress proteins, ATF6 and XBP1. Based on this result, we tested if cytisine is neuroprotective in a mouse model of striatal 6-OHDA lesions. Surprisingly, alternate day low dose i.p. injections of cytisine (0.2 mg/kg) in 6-OHDA lesioned mice reduced apomorphine rotations only in females, suggesting that cytisine requires estrogen to exert neuroprotection. Next, we assessed if estrogen inhibits CHOP, which is the downstream apoptotic protein of the third arm of the ER stress response pathway. We found that in cultured DA neurons exposed to 6-OHDA, 10 nM β-estradiol inhibited cytoplasmic expression of CHOP. Taken together, our data suggest that cytisine and estrogen exert neuroprotection in PD by synergistically inhibiting apoptotic ER stress in dopaminergic (DA) neurons.

Disclosures: S.M. Zarate: None. **G. Pandey:** None. **N.A. Salem:** None. **B. Cude:** None. **S. Storey:** None. **S. Chilukuri:** None. **J.A. Garcia:** None. **T.E. Huntington:** None. **E. Bancroft:** None. **M. Hook:** None. **R. Srinivasan:** None.

Poster

213. Parkinson's Disease: Molecular Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 213.04/E31

Topic: C.03. Parkinson's Disease

Support: HBGI Title III Grant
NSF MRI Grant

Title: Ameliorating the effects of an environmental toxin in a *drosophila* model of Parkinson's disease

Authors: *D. WILLIAMS, H. LAWAL, S. BOPPANA;
Delaware State Univ., Dover, DE

Abstract: Parkinson's Diseases (PD) is a neurodegenerative disorder characterized in part by the selective loss of dopaminergic neurons in the substantia nigra pars compacta. Although the precise cause of PD is not yet fully understood, environmental factors are known to contribute to its etiology. Rotenone, a pesticide that inhibits Complex 1 of the Electron Transport Chain in the mitochondria, is one such toxin. Importantly, there is no known cure for PD and effective treatment options are severely limited both in number and efficacy. We are interested in developing neuroprotective strategies that may lead to more effective treatments for the disease. This project will study the effects of rotenone-induced toxicity in adult *Drosophila melanogaster* and the neuroprotective capacity of dacarbazine, a possible anti-PD drug that was identified in a previous pharmacological screen. We hypothesized that dacarbazine will confer both organismal and neuroprotection against rotenone-induced toxicity and mitochondrial dysfunction. And we report that treatment with dacarbazine led to a partial rescue of organismal lethality induced by rotenone. Moreover, we report the findings of our study to determine whether the protection conferred by dacarbazine against lethality extends to the protection of DA neurons in *Drosophila*. Further, we measured the effect of rotenone on mitochondrial oxygen consumption rate using the Seahorse Analyzer and tested whether treatment with dacarbazine can ameliorate the effects of the rotenone inhibition of the mitochondria. Our studies are representative of three independent experiments; with each experiment containing a minimum of three biological replicates. In sum, our report shows the utility of a potential neuroprotective chemical against a model of PD and suggests a possible neuroprotective mechanism against the diseases.

Disclosures: D. Williams: None. **H. Lawal:** None. **S. Boppana:** None.

Poster

213. Parkinson's Disease: Molecular Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 213.05/E32

Topic: C.03. Parkinson's Disease

Support: NIH grant AG048918

Title: Pioglitazone increases striatal paraoxonase-2 expression: A novel neuroprotective target in Parkinson's disease

Authors: *J. K. BLACKBURN, D. CURRY, A. THOMSEN, R. ROTH, J. ELSWORTH; Yale Univ., New Haven, CT

Abstract: Mitochondrial alterations and oxidative stress contribute to the progressive loss of midbrain dopaminergic neurons observed in Parkinson's disease, however, there are currently no available treatments that target these mechanisms. Recently, peroxisome proliferator-activated receptors (PPARs) have been proposed as potential neuroprotective targets to slow down the progression of early PD. PPARs are members of the nuclear receptor superfamily that regulate gene expression via peroxisome proliferator response elements. The type 2 diabetes mellitus drug and PPARγ activator, pioglitazone, has recently been shown to be neuroprotective in PD models by reducing oxidative stress and/or inflammation, but its precise mechanism is not yet known.

PPARy regulates expression of paraoxonase-2 (PON2), a critical enzyme necessary for proper mitochondrial function, which enhances the function of the essential cofactor coenzyme Q in the mitochondrial electron-transport chain and reduces the production of reactive oxygen species that lead to oxidative stress. PON2 is the only isoform of the enzyme that is present in brain and it is most highly expressed in dopamine-rich regions of mouse brain (Costa et al. 2014. Toxicology 43: 3-9). Our lab has recently identified PON2 as a 'juvenile protection factor' that is highly expressed in the infant brain, potentially explaining our observation of early-life resistance to the impact of oxidative stress typically induced in dopamine neurons by parkinsonian toxins. We therefore examined whether pioglitazone can alter PON2 expression and found that sub-chronic pioglitazone significantly increased striatal expression of PON2 by 36% in mice. Ongoing studies are determining the regional distribution of PON2 and impact of pioglitazone on PON2 expression in nonhuman primate brain (St Kitts African green monkeys) and whether an increase in PON2 expression is associated with protection of the nigrostriatal dopamine system and changes in oxidative stress markers. As PD is 1.5-times more frequent in females than males, it is interesting that our data also reveal a sex difference in PON2 expression in primate brain, with significantly higher levels present in female striatum than in males. Administration of the aromatase inhibitor letrozole revealed the extent to which PON2

expression and activity is regulated by estrogen.
This report provides evidence for a novel mechanism by which pioglitazone provides neuroprotection. These findings expand the limited understanding of the properties of pioglitazone and suggest a new target for providing neuroprotection against the progression of PD.

Disclosures: J.K. Blackburn: None. **D. Curry:** None. **A. Thomsen:** None. **R. Roth:** None. **J. Elsworth:** None.

Poster

213. Parkinson's Disease: Molecular Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 213.06/E33

Topic: C.03. Parkinson’s Disease

Support: NS100090
NS088206
ES026892
Lloyd Endowment
Armbrust Endowment
Salisbury Endowment

Title: CSF-1R promotes neuroinflammation through FYN-NLRP3 signaling axis in experimental Parkinson's disease

Authors: M. SAMIDURAI, N. KONDRU, E. MALOVIC, H. JIN, A. VELLAREDDY, *A. KANTHASAMY, A. KANTHASAMY;
Iowa State Univ., AMES, IA

Abstract: A growing body of evidence suggests a major role for microglia mediated neuroinflammatory response in Parkinson’s disease (PD) associated dopaminergic neurodegeneration. Moreover, aggregated alpha-synuclein (a-syn), a major component of Lewy body has been found in the vicinity of activated microglia in PD vulnerable brain regions. Despite this, the functional interaction between a-syn and microglial activation response remains poorly characterized. In the present study, we report CSF-1R-FYN-NLRP3 signaling plays a role in a-syn induced neuroinflammation and neurodegeneration in preclinical models of PD. First, a-syn PFF-induced microglial ROS generation, nitric oxide production, and extracellular release of proinflammatory cytokines- IL-1B, and TNF-alpha. Second, asyn PFF-induced proinflammatory response was associated with the uptake of aggregated a-syn and concomitant activation of FYN-NLRP3 signaling axis in microglial cells. Importantly, the conditioned media harvested from a-syn PFF treated microglial cells elicited N27 dopaminergic neuronal cell death as compared to

vehicle treated cells. In contrast, a CSF-1R antagonist PLX5622 attenuated a-syn PFF induced microglial proinflammatory response, the uptake of aggregated alpha-syn and the activation of FYN-NLRP3 signaling axis in microglial cells. Additionally, PLX5622 reduced dopaminergic cell death elicited by activated microglia, demonstrating a role for microglial CSF-1R in dopaminergic neurodegeneration. Notably, our in vivo studies revealed that dietary PLX5622 rescued behavioral impairments in an a-syn PFF mouse model of PD. Our studies identify CSF-1R as a potential regulator of-asyn-uptake and resultant proinflammatory response and suggest that activation of microglial FYN-NLRP3 signaling axis may serve as a potential neuropathological intermediate in PD and related synucleinopathies.

Disclosures: M. Samidurai: None. **N. Kondru:** None. **E. Malovic:** None. **H. Jin:** None. **A. Vellareddy:** None. **A. Kanthasamy:** None. **A. Kanthasamy:** None.

Poster

213. Parkinson's Disease: Molecular Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 213.07/E34

Topic: C.03. Parkinson’s Disease

Support: NINDS R01NS095374
NIDA T32DA043469

Title: Synaptic mechanisms underlying caffeine’s protective effects in Parkinson’s disease

Authors: *M. J. PATEL[1], J. KORANDA[1], A. C. KROK[2], L. MO[1], X. ZHUANG[1];
[1]Neurobio., Univ. of Chicago, Chicago, IL; [2]New York Univ., New York, NY

Abstract: Chronic consumption of caffeine, an A_{2A} adenosine receptor antagonist, has long been considered to protect against the development of Parkinson’s disease. However, the mechanisms underlying this protection remain largely unknown. To examine the effect of caffeine on parkinsonian motor suppression, we trained chronically caffeine-treated mice on an accelerating rotarod while under dopamine receptor blockade. Previous work from our laboratory has shown that the combination of dopamine receptor blockade with concurrent motor experience results in long-lasting, aberrant motor inhibition. Here, we found that mice previously treated with chronic caffeine were protected from subsequent aberrant motor inhibition. We also found that theophylline, another A_{2A} antagonist, protected mice against haloperidol-induced sensitization of catalepsy when co-administered with haloperidol. To examine the synaptic mechanisms underlying this protection, we used whole-cell recordings to measure changes in corticostriatal plasticity following the onset of aberrant motor inhibition. We found that rotarod training under D2 dopamine receptor blockade resulted in abnormal strengthening of corticostriatal synapses from D2-expressing medium spiny neurons. Furthermore, prior co-administration of theophylline

was sufficient to block this aberrant plasticity. These results implicate the modulation of corticostriatal plasticity in the protective effects of caffeine against Parkinson's disease.

Disclosures: **M.J. Patel:** None. **J. Koranda:** None. **A.C. Krok:** None. **X. Zhuang:** None. **L. Mo:** None.

Poster

213. Parkinson's Disease: Molecular Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 213.08/E35

Topic: C.03. Parkinson's Disease

Support: Qilu Pharmaceutical Co., Ltd.

Title: GM1 ganglioside as a modifier of alpha-synuclein toxicity *in vivo* and in cell culture

Authors: *V. SINGH, R. ARAS, G. SINGH, J. SCHNEIDER;
Pathology, Anatomy, and Cell Biol., Thomas Jefferson Univ., Philadelphia, PA

Abstract: Parkinson's disease (PD) is a neurodegenerative disorder characterized by loss of dopamine (DA)-producing neurons in the substantia nigra (SN), decreased levels of DA primarily in the caudate nucleus and putamen, and accumulation of insoluble α-synuclein (a-Syn) aggregates. Alpha-synuclein oligomerization and aggregation are important contributors to PD pathophysiology, with a-Syn-containing cytoplasmic inclusions a histological hallmark of the disease. We recently reported that in an AAV-A53T a-Syn rat model, GM1 ganglioside administration protected against a-Syn toxicity and development of PD-relevant pathological changes and behavioral deficits. Additional studies have been performed to better understand potential mechanisms through which GM1 exerts these positive effects. In vitro studies with a-Syn expressing cell lines and in vivo studies with AAV-A53T a-Syn rats have been performed to examine the extent to which GM1 may lower overall levels of a-Syn, inhibit a-Syn aggregation, influence intracellular degradation of a-Syn, influence extracellular degradation of a-Syn, and/or influence a-Syn uptake by neighboring cells. In the AAV-A53T a-Syn rat, GM1 administration resulted in a slight increase in levels of monomeric a-Syn in the SN, perhaps signaling a shift from aggregated to monomeric a-Syn in these animals. Levels of autophagic markers p62 SQSTM and Beclin 1 in the SN suggest decreased autophagy in AAV-A53T a-Syn rats and an increase in autophagy in GM1-treated animals. RET signaling was impaired in AAV-A53T a-Syn rats and rescued by GM1 treatment. In an inducible a-Syn SH-SY5Y cell line (gift from M.L. Hegde) or stably a-Syn-expressing MN9D cells (gift from A.G. Kanthasamy), incubation of cells in GM1 for 24 hrs reduced levels of aggregated a-Syn. Normal SH-SY5Y cells took up a-Syn from conditioned media (CM) taken from a-Syn expressing MN9D cells. Incubation of SH-SY5Y cells with GM1 at least partially inhibited accumulation of a-Syn from CM from a-

Syn expressing MN9D cells, suggesting a potential effect on a-Syn uptake. Studies are also investigating the extent to which GM1 may influence the release of a-Syn from cells. Together, these data suggest an important role for GM1 in modulating various aspects of a-Syn toxicity, which could have important implications for the treatment of PD.

Disclosures: V. Singh: None. **R. Aras:** None. **G. Singh:** None. **J. Schneider:** None.

Poster

213. Parkinson's Disease: Molecular Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 213.09/E36

Topic: C.03. Parkinson's Disease

Title: Improved cell-permeable (iCP)-Parkin has neuro-cytoprotective activity by inducing mitophagy and mitochondria biogenesis

Authors: *W. NAH, E. CHUNG, J. PARK, S. PARK, S. KIM, W. LEE, H. CHO, Y. CHOI, D. JO;
Cellivery R&D Inst., Seoul, Korea, Republic of

Abstract: Parkinson's disease (PD) is a neurodegenerative disorder that causes abnormal movement due to the selective loss of dopaminergic (DA) neurons in the substantia nigra of the brain. Parkin is an E3 ubiquitin ligase that plays a critical role in the ubiquitination process. When mitochondrial damage occurs, PINK1 is recruited to the mitochondrial membrane and phosphorylates Ser-65 of Parkin which induces conformational change of its structure to be active. Phosphorylated and activated Parkin ubiquitinates various substrates and induces mitophagy in damaged mitochondria. Improved cell-permeable (iCP) Parkin fused to an advanced macromolecule transduction domain (aMTD) has been developed and produced by taking denaturation and refolding process. Constitutively active iCP-Parkin does not require phosphorylation of Ser-78 by PINK1 for its activity. It may suggest that this fusion recombinant Parkin protein possesses open conformational structure to be active regardless of PINK1-mediated Ser-78 phosphorylation. iCP-Parkin is accumulated in damaged mitochondria and ubiquitinates various substrates involved in mitophagy (MFN1/2 and VDAC1), mitochondrial transport (Miro1/2) and mitochondrial biogenesis (PARIS). iCP-Parkin also induces expression of mitochondrial proteins such as Cox-1, SDH-A, PGC-1α, TFAM and NRF2, which are involved in mitochondria biogenesis. In conclusion, iCP-Parkin recovers mitochondria functionality by utilizing its ubiquitin E3 ligase activity and rescues neurons under PD-like pathological conditions.

Disclosures: W. Nah: None. **E. Chung:** None. **J. Park:** None. **S. Park:** None. **S. Kim:** None. **W. Lee:** None. **H. Cho:** None. **Y. Choi:** None. **D. Jo:** None.

Poster

213. Parkinson's Disease: Molecular Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 213.10/E37

Topic: C.03. Parkinson's Disease

Title: Transcriptomic analysis of zebrafish CDNF mutant brains

Authors: *Y.-C. CHEN[1], J. T. LEINONEN[2], E. WIDEN[2], P. PANULA[1];
[2]Inst. for Mol. Med. Finland (FIMM), [1]Univ. of Helsinki, Helsinki, Finland

Abstract: Cerebral dopamine neurotrophic factor (CDNF) together with mesencephalic astrocyte-derived neurotrophic factor (MANF) belong to the unconventional neurotrophic factor family because their structures and functions differ from classic target-derived neurotrophic factors. Growing evidence shows that CDNF provides neurorestorative and protective effects against endoplasmic reticulum stress possibly by means of its anti-apoptotic, anti-inflammatory and antioxidative properties. However, the precise molecular mechanisms behind protective actions of CDNF are unknown. We have previously generated *cdnf* mutant zebrafish and reported that these fish developed social impairments that may be associated with deficiencies in the dopaminergic, GABAergic and histaminergic circuits in certain brain areas. In this study, we utilized RNA-sequencing to further characterize the molecular changes caused by *cdnf* knockout. We generated RNA-seq data from adult brains of 6-month-old *cdnf* mutant and wild-type (WT) sibling (N=5 per group). The differential expression (DE) analysis showed that 800 genes were differently expressed in *cdnf* mutant brains compared to *cdnf* WT fish ($p<0.05$). Of 464 up-regulated genes, 10 genes were upregulated more than 4- fold (FDR-adjusted p-value $q< 0.1$) and three of these were identified as regulators of chemokine activity, indicating that zebrafish *cdnf* may play a functional role in inflammatory response. On the other hand, out of 336 down-regulated genes, eight genes altered more than 4-fold ($q<0.1$) were defined to have hydrolase activity, function in cell-cell adhesion or integrate into the hydrophobic region of the membrane, suggesting that cdnf may be involved in the regulation of membrane protein transport. Taken together, our whole brain transcriptomic dataset of the *cdnf* mutant zebrafish may provide a considerable resource for understanding CDNF functions underlying the neuroprotective pathway.

Disclosures: Y. Chen: None. **J.T. Leinonen:** None. **E. Widen:** None. **P. Panula:** None.

Poster

213. Parkinson's Disease: Molecular Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 213.11/E38

Topic: C.03. Parkinson's Disease

Title: Cell-permeable parkin suppresses accumulation of pathological alpha-synuclein

Authors: E. CHUNG, ***M. JUNG**, H. LEE, S. KIM, S. HWANG, J. JO, Y. CHOI, G. KIM, Y. CHOI, D. JO;
Cellivery Therapeutics, Inc., Seoul, Korea, Republic of

Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by dopaminergic cell loss in the substantia nigra and formation of Lewy body aggregates. Approximately 90% of Lewy body is consisted of Ser-129 phosphorylated α-Synuclein. Parkin, an E3 ubiquitin ligase, is able to remove damaged organelles and aggregated proteins. To clear pathological α-Synuclein aggregates, improved cell-permeable (iCP) Parkin recombinant protein has been developed by fusing to advanced macromolecule transduction domain (aMTD). In α-Synuclein-overexpressing cells treated with neuro-toxin to induce pathological (oligomeric or filamentous, and phosphorylated) forms of α-Synuclein, intracellularly-delivered iCP-Parkin suppresses pathogenic α-Synuclein, and also shows a modified autophagic flux. As an E3 ubiquitin ligase, iCP-Parkin is able to ubiquitinate substrates such as Parkin-related endothelin receptor-like receptor (Pael-R) and Synuclein-alpha-interacting protein (Synphilin-1), which are involved in formation of Lewy body. In conclusion, iCP-Parkin induces cytoprotective effect by ubiquitinating multiple Lewy body relevant substrates and reducing pathological forms of α-Synuclein. Based on these results, we suggest that iCP-Parkin works not only as a suppressor for pathological α-Synuclein but also as an anti-apoptotic neural savior, a potential drug candidate for Parkinson's disease.

Disclosures: E. Chung: None. **M. Jung:** None. **H. Lee:** None. **S. Kim:** None. **S. Hwang:** None. **J. Jo:** None. **Y. Choi:** None. **G. Kim:** None. **Y. Choi:** None. **D. Jo:** None.

Poster

213. Parkinson's Disease: Molecular Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 213.12/E39

Topic: C.03. Parkinson's Disease

Support: SERB-DST
AICTE
UGC

Title: Activation of Nrf2/ARE pathway as a potential mechanism for therapeutic effect of NDGA against 6-OHDA-induced Parkinson's disease in mice

Authors: *R. K. SODHI, Y. BANSAL, R. SINGH, P. SAROJ, A. KUHAD;
Pharmacol., Univ. Inst. of Pharmaceut. Sci., Chandigarh, India

Abstract: Introduction: Oxido-inflammatory aberrations in conjunction with mitochondrial dysfunction play a substantial role in the pathophysiology of Parkinson's disease (PD). Oxidative stress triggers inflammatory response and apoptosis leading to dopaminergic cell death causing PD. Antioxidants have shown potential as pharmacotherapeutic agents for the treatment of PD. Nordihydroguaiaretic acid (NDGA), an Nrf2 activator has shown positive effects in PD in *Drosophila* model and has also shown to act by activating the Nrf2/ARE pathway but whether its protective effect in PD is due to activation of Nrf2/ARE pathway or not still remains to be explicated. With this background, the existing study was designed to explore the involvement of Nrf2/ARE pathway as a pharmacotherapeutic mechanism of NDGA in 6-OHDA-induced mouse model of PD.
Materials and Methods: Male BALB/c mice were injected 6-OHDA (3µg/µL) directly into the striatum which induced Parkinson's like symptoms as measured by reduced mitochondrial complex activity (complex I and IV), increased oxidative stress (malonyldialdehyde and plasma nitrite), reduced endogenous antioxidant enzymes (superoxide dismutase, catalase, reduced glutathione, Nrf2, HO-1), increased apoptosis (caspase-3), increased striatal neuroinflammation (NF-κB, IL-1β, TNF-α, IFNϒ), decreased striatal neurotransmitter levels (dopamine and homovanillic acid) and locomotor deficits (Rota-rod and open field test) in mice in comparison to sham control after 14 days of injection.
Results: Two weeks per oral treatment with NDGA (7.5, 15 and 30 mg/kg) reversed the locomotor deficits, restored the endogenous antioxidant enzyme (SOD, catalase, GSH, HO-1, Nrf2) levels, reduced oxido-nitrosative stress (decreased MDA and nitrite), prevented mitochondrial dysfunction and apoptosis. It also increased the neurotropic factor BDNF and reduced cytokine transcription factor NF-κB along with inflammatory cytokines like IL-1β, TNF-α, IFNϒ. NDGA was also found to restore dopamine and homovanillic acid levels in the mice striatum which were decreased after 6-OHDA injection.
Conclusion: In conclusion, NDGA showed potent neuroprotective effect in 6-OHDA-induced PD in mice by means of its antioxidant and anti-inflammatory potential by activating the Nrf2/ARE pathway.

Disclosures: R.K. Sodhi: None. **Y. Bansal:** None. **R. Singh:** None. **P. Saroj:** None. **A. Kuhad:** None.

Poster

213. Parkinson's Disease: Molecular Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 213.13/E40

Topic: C.03. Parkinson’s Disease

Support: NRF-2017R1A2B4008456

Title: The effects of valproic acid on neuroinflammation in LRRK2 R1441G mice

Authors: *Y. PARK, S. SONG, T. KIM, H. NOH, S. KANG, H. SEO;
Hanyang Univ., Seoul, Korea, Republic of

Abstract: Numerous studies have shown that activated microglia release pro-inflammatory markers and results in dopaminergic neuronal degeneration in Parkinson’s disease (PD). Some of histone deacetylase (HDAC) inhibitors are reported neuroprotective in various neurodegenerative diseases. We previously studied that valproic acid (VPA), pan inhibitor of HDAC, alleviated neuroinflammation in Alzheimer’s disease. In this study, we hypothesized that HDAC inhibition decrease the expression of pro-inflammatory markers and increased the survival of dopaminergic neurons in PD model. To determine the effects of VPA on neuroninflammation, the activated microglial cells in substantia nigra (SN) and striatum (ST) were counted by immunohistochemical staining using anti-Iba-1 antibody. Consequently, we found that VPA decreased the number of activated microglia in ST of LRRK2R1441G mice and altered the histone acetylation related expression levels of pro-inflammatory markers including Fc gamma receptors (FcγRs) in LRRK2 R1441G mice and LPS-induced activated microglia, BV2 cells. In addition, VPA improved non-motor PD symptoms, which were detected by time spent in center from open field test and cognitive behaviors from elevated plus maze test. These data suggest that the regulation of histone acetylation through neuroinflammatory responses can be future therapeutic target of PD to recover not only nigrostriatal pathway but also mesocorticolimbic pathway of dopaminergic system.

Disclosures: Y. Park: None. **S. Song:** None. **T. Kim:** None. **H. Noh:** None. **S. Kang:** None. **H. Seo:** None.

Poster

213. Parkinson's Disease: Molecular Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 213.14/E41

Topic: C.03. Parkinson's Disease

Support: KHIDI Grant HI16C1012
KHIDI Grant HI16C1176

Title: Erythropoietin and granulocyte colony stimulating factor co-treatment restores impaired behaviors in Parkinson's disease

Authors: *E. CHO, H. KIM, S. PYO, S. JO, S. SONG, B. NAM, J. HEO, J. YU, Y. SHIN, S. CHO;
YONSEI UNIVERSITY, Seoul, Korea, Republic of

Abstract: Parkinson's disease (PD) is one of major neurodegenerative disorders, characterized by the decreasing number of dopamine neurons in the substantia nigra pars compacta (SNpc) and appearing α-synuclein aggregations called Lewy bodies. In this study, we aimed to determine whether erythropoietin (EPO) and granulocyte colony stimulating factor (GCSF) co-treatment has beneficial effects on dopamine neuron protection and improvement on motor behavior functions. Our results showed that, *in vitro*, dopamine neuron cell viability increased by 73% in differentiated mouse N2A cells and by 84% in human derived SHSY5Y cells with EPO and GCSF co-treatment than with EPO or GCSF alone against MPP^+(2.5mM for 24-hr) neurotoxin. *In vivo* study, we subcutaneously injected EPO and/or GCSF for 5 consecutive days (one time/a day) into MPTP (20mg/kg, 2-hr interval, 4 times for a day) intraperitoneal injection mice. We observed that increased tyrosine hydroxylase(TH^+) fiber density in the striatum (STR) as well as amounted number of TH^+ cells in the SNpc in EPO and GCSF co-injected mice group than either EPO or GCSF injected mice group. We also observed an enhanced locomotor function with EPO and GCSF subcutaneous co-injection mice group than that of EPO or GCSF alone, confirmed by rota-rod treadmill test (latency to fall), ladder walking test (the number of reciprocal walking), hanging wire test (time of grip hold), and open field test (distance). To sum up, our results suggested EPO and GCSF co-treatment not only restores impaired locomotor function but also protects dopamine neuron against MPP^+ and MPTP neurotoxins *in vitro* and *in vivo*, respectively. Therefore, treatment with both EPO and GCSF rather than with EPO or GCSF alone could be a promising therapeutic approach for PD.
Acknowledgement: This study was supported by grants from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI) funded by the Ministry of Health and Welfare (HI16C1012, HI16C1176), South Korea.

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Poster

213. Parkinson's Disease: Molecular Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 213.15/E42

Topic: C.03. Parkinson's Disease

Support: Parkinson Support Group

Title: Oxr1 protects against neurodegeneration in experimental Parkinson's disease

Authors: *N. AMMAL KAIDERY[1,2], M. AHUJA[1,2], P. L. OLIVER[5,6], B. THOMAS[1,2,3,4]; [1]Darby Res. Inst., [2]Pediatrics, [3]Neurosci., [4]Drug Discovery, Med. Univ. of South Carolina, Charleston, SC; [5]Dept. of Physiology, Anat. and Genet., Univ. of Oxford, Oxford, United Kingdom; [6]MRC Mammalian Genet. Unit, MRC Harwell Inst., Oxford, United Kingdom

Abstract: Parkinson's disease (PD) is the most common neurodegenerative movement disorder characterized by loss of nigrostriatal dopaminergic neurons affecting motor functions. Oxidative stress is a common etiological feature of PD, although the pathways that govern defense against reactive oxygen species in neurodegeneration remain unclear. Oxidation resistance 1 (Oxr1) controls the sensitivity in neuronal cells to oxidative stress through unknown mechanisms. We investigated the role of Oxr1 in mediating intrinsic protective pathways in neurons using cellular and mouse models of PD. Confocal microscopy, immunoblot, and realtime RT-PCR analysis of the mouse midbrain suggests that Oxr1 is expressed in substantia nigra pars compacta (SNpc) dopaminergic neurons. Oxr1 expression is upregulated in cellular and mouse models of PD as an early marker of stress in neurons. Sub-cellular fractionation, confocal microscopy and immunoblot analysis showed that Oxr1 is predominantly cytosolic with a punctate distribution and co-immunoprecipitated with subunits of lysosomal vacuolar ATPase. SiRNA knockdown of Oxr1 in Neuro-2A cells increased lysosomal pH, decreased activities of lysosomal enzymes, and increased sensitivity to rotenone and MPP+ (1-methyl-4-phenyl pyridinium ion) toxicity compared controls. To determine the role of Oxr1 in neurodegeneration as seen in PD, we examined dopaminergic neurotoxicity in wild type and transgenic mice expressing Oxr1 under mouse *Prnp* promoter using the parkinsonian neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). Transgenic Oxr1 mice were more resistant to acute and sub-acute regimens of MPTP-neurotoxicity compared to wild type controls as analyzed by unbiased stereological counts of tyrosine hydroxylase-positive neurons of SNpc. This resistant phenotype was not due to impaired metabolism or trafficking of MPP+ (1-methyl-4-phenylpyridinium ion), the toxic metabolite of MPTP. Markers of inflammatory response and oxidative stress post MPTP treatment was significantly attenuated in Oxr1 transgenic mice as measured by expression levels

of TNF-α, MCP-1 and CD68 using realtime PCR and 3-nitrotyrosine by immunohistochemistry. Our results provide evidence for a novel role of Oxr1 in neuroprotection and may serve as a promising target for therapeutic intervention in PD.

Disclosures: **N. Ammal Kaidery:** None. **M. Ahuja:** None. **P.L. Oliver:** None. **B. Thomas:** None.

Poster

213. Parkinson's Disease: Molecular Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 213.16/E43

Topic: C.03. Parkinson's Disease

Title: Uric acid enhances neurogenesis in Parkinsonian model

Authors: *J. LEE[1], J. SHIN[2], H. KIM[2], D. KIM[2], P. LEE[2];
[1]Neurol., Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; [2]Yonsei Univ., Seoul, Korea, Republic of

Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disease characterized by the selective loss of dopaminergic neurons in the substantia nigra (SN) and the degeneration of projecting nerve fibers to the striatum, which leads to motor disorder such as bradykinesia, rigidity, and tremor. Though multiple factors are underlying PD pathogenesis, oxidative stress and mitochondrial dysfunction is the main pathology in PD. Another striking symptom is that neurogenesis is significantly reduced in PD patients. Neurogenesis is the ability of brain to generate new neurons throughout whole life even after embryo development. This process mainly occurs in two regions: the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus in the hippocampus. Neurogenesis in SVZ is decreased in PD, thus the opportunity to differentiate into functional neurons decreases as well, which leads to PD symptom and restoring it could be one potential strategy. Interestingly emerging clinical data have reported the association between PD and uric acid (UA) levels. The meta-analysis results showed that PD patients have lower UA levels than healthy controls and high blood levels of UA are associated with reduced risk of developing PD. UA is a natural powerful antioxidant, which has neuroprotective effect on PD by scavenging oxygen radicals and reducing oxidative stress. This study is aimed to investigate the link between neurogenesis and UA level in PD. To evaluate the effect of UA on neurogenesis, 5weeks old male C57BL/6 mice were divided into four groups; control, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), MPTP+UA post-treatment, MPTP+UA pre-treatment. Uricase inhibitor and inosinic acid was given to increase serum UA level. Post-treatment group received four weeks of daily injection after MPTP induction and pre-treatment group got two weeks of daily injection prior to MPTP induction and received additional four weeks of injection. Previous studies showed that

neurogenesis in SVZ of MPTP-induced parkinsonian model was significantly decreased compared to that of wild type. In this study, though there was no difference in post-treatment model, neurogenesis in UA pre-treatment group was significantly restored by 41%(±0.05). This result suggests UA could be a candidate drug as it increases the number of newborn neurons, which will migrate, enter neural circuit, and differentiate into functional mature neurons, finally slowing down progression of PD or even prevent it.

Disclosures: **J. Lee:** None. **J. Shin:** None. **H. Kim:** None. **D. Kim:** None. **P. Lee:** None.

Poster

213. Parkinson's Disease: Molecular Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 213.17/E44

Topic: C.03. Parkinson's Disease

Support: Parkinson Society Canada 106383

Title: The neuroprotective effects of optogenetic stimulation of astroglia in a rat model of neurodegeneration

Authors: *J. L. MCNEILL[1], G. COPPOLA[2], C. A. RUDYK[1], I. TRUJILLO-PISANTY[3], K. FARMER[4], N. SALMASO[1];
[1]Neurosci., Carleton Univ., Ottawa, ON, Canada; [2]Child Study Ctr., Yale Univ., New Haven, CT; [3]Psychiatry and Neurosci. Ctr., Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; [4]Neurol., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disease, estimated to affect approximately 6.1 million individuals worldwide. PD is characterized by progressive degeneration of dopaminergic neurons within the substantia nigra pars compacta (SNc) and the widespread accumulation of α-synuclein plaques. Precise mechanisms underlying this continuous loss of dopaminergic neurons remain largely elusive, as do effective treatments for PD. Historically, astroglial cells have been examined in PD in the context of reactive injury, however recent studies suggest that they may be casually involved and potential therapeutic targets. To further elucidate the therapeutic potential of astroglia, we used optogenetics to stimulate astroglia in the SNc of rats injected with 6-OHDA, which induces dopaminergic degeneration. Four groups of rats were employed to study this: 1) Sham lesion; Sham stimulation 2) 6-OHDA lesion; Sham stimulation 3) Sham lesion; Stimulation 4) 6-OHDA lesion; Stimulation. One week following the 6-OHDA lesion (or sham control) and post-stimulation (or sham stimulation), animals underwent a battery of behavioural tests and brains were harvested for histological analysis. As predicted, lesioned animals showed significant motor deficits related to dopamine cell loss. Excitingly, astroglial stimulation was sufficient to reverse all of these

behavioural deficits. To further understand the potential neuroprotective effects of optogenetically stimulating astroglia, whole tissue RNA-sequencing of the SNc was conducted on a group of behaviourally-naïve rats. We assessed differentially expressed genes and also conducted gene set enrichment analysis. The top gene sets implicated in lesion that were reversed with stimulation were genes associated with immune pathways, cell surface membrane proteins and GPCR signaling. Together, these results affirm the potential for astroglia as therapeutic targets in PD and potentially identify mechanistic targets for future validation.

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Poster

213. Parkinson's Disease: Molecular Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 213.18/F1

Topic: C.03. Parkinson’s Disease

Support: ANPCyT PICT2016 0588
INYM PRASY 2016

Title: Neuroprotective effect of Yerba Mate (ilex paraguariensis) intake on dopaminergic neurons in a mice model of Parkinson's disease

Authors: *G. GOMEZ[1], L. T. TRIBBIA[2], A. C. CURA[2], R. RIVERO[2], M. A. BERNARDI[3], J. E. FERRARIO[1], B. BALDI-CORONEL[4], O. S. GERSHANIK[1], E. GATTO[5], I. R. E. TARAVINI[2];
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Abstract: A key feature of Parkinson’s disease (PD) is the progressive loss of midbrain dopaminergic neurons and their axons projecting mainly to the striatum. The mechanisms underlying this neuronal degeneration have not been fully characterized and there is no current preventive therapy for PD. However, an inverse association was found between coffee intake or smoking and the occurrence of PD. The infusion from the plant Ilex paraguariensis (popularly known as Yerba mate, YM) produces a very popular South American and Mediterranean beverage called ‘mate’. This infusion contains several bioactive phenolic compounds, which are antioxidant and anti-inflammatory. A case-control study revealed that consumption of mate also

has an inverse association with the risk of developing PD. Furthermore, we have recently shown that YM favors survival and growth of dopaminergic neurons in culture. In the present study, we investigated the possible neuroprotective effect of YM intake on dopaminergic neurons in the mice model of PD induced by 6-hydroxydopamine (6-OHDA) injection. As YM infusion is not commonly made as an herb tea, we used an extraction method called 'cebada simulada' that resembles the way it is usually consumed. We quantified the main bioactive compounds (caffeine, theobromine, chlorogenic acid and rutin) by HPLC. Mice received water (control) or 'mate' as their only source of fluid, and different periods of YM administration and concentrations were evaluated. The infusion was well accepted by the animals, showing no difference in the total volume consumed compared with water. Locomotor activity was evaluated in open field sessions and we observed that mice that drank mate had a hyperlocomotor behavior. To induce a partial degeneration of dopaminergic neurons as an early PD model, 6-OHDA was injected unilaterally into the striatum using different coordinates, volumes and concentrations. After sacrifice, Tyrosine hydroxylase (TH) immunohistochemistry was performed to evaluate the degree of dopaminergic denervation. We developed a protocol of denervation which induced a lesion level between 30-60 percent. Our histological data showed that YM intake increases the TH immunoreactive remaining fibers compared to control mice, suggesting a neuroprotective effect against 6-OHDA neurotoxicity.

Disclosures: G. Gomez: None. **L.T. Tribbia:** None. **A.C. Cura:** None. **R. Rivero:** None. **M.A. Bernardi:** None. **J.E. Ferrario:** None. **B. Baldi-Coronel:** None. **O.S. Gershanik:** None. **E. Gatto:** None. **I.R.E. Taravini:** None.

Poster

213. Parkinson's Disease: Molecular Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 213.19/F2

Topic: C.03. Parkinson's Disease

Support: Merit Award U.S. Department of Veterans Affairs (I01BX003033)
NIH Grant NS108025

Title: Gemfibrozil protects dopaminergic neurons in a mouse model of Parkinson's disease via a PPAR alpha-dependent astrocytic GDNF pathway

Authors: *C. G. GOTTSCHALK[1], A. ROY[2], K. PAHAN[3];
[1]Neurolog. Sci., Rush Univ., Chicago, IL; [3]Dept Neurolog. Sci., [2]Rush Univ. Med. Ctr., Chicago, IL

Abstract: Parkinson's disease (PD) is the most common neurodegenerative movement disorder in human. Despite intense investigations, effective therapies are not yet available to halt the

progression of PD. Gemfibrozil, an FDA-approved lipid-lowering drug, is known to decrease the risk of coronary heart disease by increasing the level of high density lipoprotein cholesterol and decreasing the level of low density lipoprotein cholesterol. This study underlines the importance of gemfibrozil in protecting dopaminergic neurons in an animal model of PD. Oral administration of human equivalent dose of gemfibrozil protected tyrosine hydroxylase (TH)-positive dopaminergic neurons in the substantia nigra pars compacta (SNpc) and TH fibers in the striatum of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-insulted mice. Accordingly, gemfibrozil also normalized striatal neurotransmitters and improved locomotor activities in MPTP-intoxicated mice. Gemfibrozil-mediated protection of the nigrostriatum and locomotor activities in WT and PPARβ (-/-), but not PPARα (-/-), mice from MPTP intoxication suggests that gemfibrozil needs the involvement of PPARα in protecting dopaminergic neurons. While investigating further mechanisms, we found that gemfibrozil stimulated the level of glial-derived neurotrophic factor (GDNF) in astrocytes via PPARα and that gemfibrozil protected nigral neurons, normalized striatal fibers and neurotransmitters, and improved locomotor activities in MPTP-intoxicated G*fap*^{cre} mice, but not *Gdnf*$^{\Delta astro}$ mice lacking GDNF in astrocytes. These findings highlight the importance of PPARα-dependent astroglial GDNF pathway in gemfibrozil-mediated protection of dopaminergic neurons in an animal model of PD and suggest possible therapeutic use of gemfibrozil in PD patients

Disclosures: C.G. Gottschalk: None. **A. Roy:** None. **K. Pahan:** None.

Poster

213. Parkinson's Disease: Molecular Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 213.20/F3

Topic: C.03. Parkinson’s Disease

Support: TATA Trusts Grant (JTTO0001)

Title: RNAseq analysis of the SNpc in Parkinson’s disease reveals down-regulation of mid-brain development and differentiation genes

Authors: *A. VERMA[1], E. C. HIRSCH[2], V. RAVINDRANATH[1,3];
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Abstract: Selective vulnerability to degeneration of the dopaminergic (DA) neurons in substantia nigra pars compacta (SNpc) over the DA neurons in ventral tegmental area (VTA) is a characteristic feature of Parkinson’s disease (PD). PD is a multifactorial disorder and transcriptomic sequencing enables investigation of global gene expression changes for evaluating several putative pathogenic mechanisms concurrently. While several microarray studies exist on

SNpc from rodent models of PD and PD patients, given the shortcomings of microarray, transcriptomic profiling of SNpc in PD through RNA sequencing (RNAseq) is warranted to get a more comprehensive insight. The pathological mechanisms that lead to degeneration of SNpc in PD were therefore explored by examining global changes in gene expression in SNpc from PD autopsy tissues. RNAseq was performed on Illumina platform in paired end manner (100X2 bp) generating 40-45 million reads on SNpc dissected from human PD autopsy tissue and age-matched controls. The raw reads were quality checked using FastQC and adapter trimmed using Trimmomatic. Trimmed reads were aligned to human reference genome (GRCh37) using TopHat2 and counts were assigned to the genetic loci using htseq-count. The data was further analysed for differential gene expression after normalisation using DESeq2. Further, gene-set enrichment analysis and pathway analysis were performed on the data using GAGE and ClueGO. Statistically significant downregulation of genes that are markers of DA neurons such as TH, DAT, VMAT2, DDC and NURR1 was observed. Further, pathway analysis revealed alterations in genes belonging to the pathways such as dopamine metabolism, synaptic vesicle release and genes involved in DA neuron development and differentiation. Further, using qRT-PCR, we validated the down-regulation of these genes involved in development and differentiation of mid-brain DA neurons in human PD autopsy tissue and in MPTP mouse model of PD after carbidopa-levodopa treatment. While several molecular mechanisms have been implicated in PD pathogenesis, our study elucidates the importance of genes involved in development and differentiation of mid-brain DA neurons as a novel pathway involved in PD.

Disclosures: **A. Verma:** None. **E.C. Hirsch:** None. **V. Ravindranath:** None.

Poster

213. Parkinson's Disease: Molecular Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 213.21/F4

Topic: C.03. Parkinson’s Disease

Support: DST/INSPIRE/04/2018/000190

Title: Small molecule aggrephagy modulators ameliorate Parkinson's disease

Authors: *S. S N;
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Abstract: Aggrephagy is a selective autophagy and intracellular degradation process that clears the toxic protein aggregates. Accumulation of toxic protein aggregates is one of the main pathophysiological hallmarks of neurodegenerative diseases. We aim to understand the underlying cellular basis for defunct autophagy contributing to PD pathogenesis. We addressed this question through chemical biology approach to identify the novel regulators of aggrephagy.

An unbiased, phenotypic screen in yeast revealed 6-Bio and other hits as aggrephagy modulators. All aggrephagy modulators restore proteostasis by clearing toxic α-synuclein aggregates in an autophagy dependent manner in yeast and mammalian cells. 6-Bio and others dramatically enhanced autolysosome formation resulting in α-synuclein degradation. 6-Bio and other hits are neuroprotective by inducing autophagy in dopaminergic neurons of midbrain to clear toxic protein aggregates in a preclinical mice model of PD. Modulation of autophagy by 6-Bio was GSK3B dependent (PMID: 28350199). The primary target of one of the hits is an orphan nuclear receptor. Orphan nuclear receptor down regulation enhanced autophagy and its overexpression-inhibited autophagy. We showed that in a basal state orphan nuclear receptor was localized on autophagosomes, upon autophagy induction, this localization was lost with concomitant increase in autophagosomes. Our results demonstrated new cytoplasmic function of orphan nuclear receptor in regulating autophagy (PMID: 29686608). We intend to identify the target of one of the hits by click chemistry, pull down and mass spectrometry revealed the dataset of putative 145 interacting partners. Mammalian cell based aggrephagy genetic screen encompassing the knockdown of all 145 genes identified its cellular target. Secondary assays in mammalian cell based autophagic assays confirmed that the target positively regulate the way (unpublished data). Small molecules are neuroprotective in a preclinical mouse model of PD by degrading the toxic protein oligomers. An emerging concept from our studies that autophagosome-lysosome fusion rate may be a bottleneck for autophagy flux and targeting this road block may have therapeutic benefits in PD.

Disclosures: S. S n: None.

Poster

214. Ischemic Stroke II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 214.01/F5

Topic: C.08. Ischemia

Support: Spanish Ministry of Economy and Competitiveness with FEDER funds (BFU2012-32089, BFU2015-66689, RYC-2013- 12817)
Ikerbasque Startup Fund
BBVA Foundation Grant for Researchers and Cultural Creators
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Predoctoral Fellow from the Spanish Ministry of Economy and Competitiveness
Juan de la Cierva Postdoctoral Fellowship

Title: Energy failure drives microglial phagocytosis dysfunction in stroke

Authors: *A. SIERRA[1], **S. BECCARI**[1], A. PLAZA-ZABALA[1], V. SIERRA-TORRE[1], A. CARRETERO-GUILLÉN[1], S. CASTAÑO-CASTAÑO[1], T. UMEKAWA[2], A. OSMAN[2], W.

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Abstract: Microglial phagocytosis is an essential mechanism to maintain tissue homeostasis. In physiological conditions in the adult hippocampus, apoptotic cells are rapidly and efficiently phagocytosed by microglia. In response to phagocytic challenge induced by excitotoxicity or inflammation, microglia proportionally boost their phagocytic output to counteract the increased number of apoptotic cells, thus maintaining apoptosis and phagocytosis tightly coupled. However, this phagocytic potential was blocked in a mouse model of cerebral hypoxia-ischemia. Using CX3CR1-GFP and CCR2-RFP mice, in which we can discriminate resident microglia from blood-derived monocytes, we have discovered that microglial phagocytosis is strongly uncoupled from apoptosis as early as 1d after ischemia in both postnatal day 9 (P9) and 3 month old (3m) adult brains. Importantly, we have observed that this blockage occurred before blood-derived monocyte infiltration. In addition, the mechanisms underlying the microglial phagocytosis impairment were investigated using primary and organotypic hippocampal slices under oxygen nutrient deprivation/reperfusion (OND). We found that OND conditions in primary microglial cultures led to a reduction in the degradation stage, possibly due to a lysosomal dysfunction. Interestingly, in organotypic hippocampal slices the microglial phagocyctosis was impaired under OND conditions during 3 and 6h, which was rapidly recovered after 1h reperfusion. Our hypothesis is that the phagocytic blockade was the result of a reduced microglial surveillance and motility of the processes, which was assessed by 2-photon microscopy. Accordingly, microglial phagocytic potential is a novel and yet unexplored therapy to promote clearance of apoptotic cells and immunomodulation, in order to accelerate ischemic brain recovery.

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Poster

214. Ischemic Stroke II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 214.02/F6

Topic: C.08. Ischemia

Support: NIH Grant NS088058

Title: Astrocyte-derived estrogen enhances A2-type astrocyte activation and is neuroprotective after global cerebral ischemia

Authors: *J. WANG[1], G. R. SAREDDY[3], Y. LU[2], U. P. PRATAP[3], F. TANG[2], R. K. VADLAMUDI[3], D. W. BRANN[4];
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Abstract: Expression of the 17β-estradiol (E2) synthesis enzyme, aromatase has been shown to be upregulated in astrocytes in the brain after brain injury. However, the precise role and function of astrocyte derived E2 in the brain remains unclear. To better address this issue, in the current study, we generated a conditional knockout (KO) mouse model that lacks aromatase expression in astrocytes (GFAP-Aro-KO), and used global cerebral ischemia (GCI) to study the role of astrocyte-derived E2 in hippocampal neuronal injury. The results revealed a significant increase in neuronal damage and microglial activation in both male and female GFAP-Aro-KO mice hippocampus at 3 day after GCI, suggesting that astrocyte-derived E2 has a neuroprotective role after brain ischemia. When the astrocytes were analyzed, we unexpectedly detected a pronounced decrease of astrocyte activation in both the male and female GFAP-Aro-KO mice hippocampus at 3 and 7 days after GCI. This observation is consistent with RNA-seq analysis, which showed decreased expression of astrocyte activation genes in the GFAP-Aro-KO hippocampus at 24 h after GCI. Further analysis of the RNA-seq data revealed that the GFAP-Aro-KO mouse brain failed to upregulate a group of genes, which have been suggested to represent the "A2" neuroprotective signature of astrocytes, in response to GCI. In contrast, there was up-regulation of many genes representing the "A1" neurotoxic profile of astrocytes in GFAP-Aro-KO mice. The RNA-seq data was further validated by quantitative RT-PCR. Furthermore, Ingenuity Pathway Analysis of differentially expressed genes suggested that the interleukin (IL)-6 signaling pathway is among the top regulated pathways by astrocyte-derived E2, and gene set enrichment analysis revealed that GFAP-Aro-KO regulated genes were negatively correlated with the IL-6/JAK/STAT3 gene set. Following up on this observation, we measured activation of STAT3 (by phosphoralated-STAT3, pSTAT3), and found that the p-STAT3 level and the expression of STAT3 in astrocytes were significantly decreased in the hippocampus of KO mice after GCI. In conclusion, the current study demonstrates for the first time that astrocyte-derived E2 plays a crucial role in promoting astrocyte activation toward an "A2" neuroprotective profile after brain ischemia, and this regulatory effect is likely linked to the STAT3 signaling pathway.

Disclosures: J. Wang: None. **G.R. Sareddy:** None. **Y. Lu:** None. **U.P. Pratap:** None. **F. Tang:** None. **R.K. Vadlamudi:** None. **D.W. Brann:** None.

Poster

214. Ischemic Stroke II

Location: Hall A

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Program #/Poster #: 214.03/F7

Topic: C.08. Ischemia

Support: NIH Grant NS106901
NIH Grant GM109089

Title: A single spreading depolarization can induce synaptic synaptic strengthening and BDNF upregulation

Authors: *J. WEISEND[1], K. M. REINHART[1], A. P. CARLSON[2], C. W. SHUTTLEWORTH[1]; [1]Neurosciences, [2]Neurosurg., Univ. of New Mexico, Albuquerque, NM

Abstract: Introduction: Spreading depolarizations (SDs) are slowly progressing waves of extended depolarization, and have emerged as a principal mechanism of infarct expansion. Previous work has described synaptic potentiation following SD, although the underlying mechanisms, duration and significance for pathophysiological states remain largely unknown. Increases in BDNF expression have been reported following repetitive SDs but the time course after a single SD has not been described. A mechanism coupling BDNF to synaptic strengthening is concomitant adenosine A2 receptor activation, which is of interest here because of large adenosine accumulations following a single SD. We therefore tested effects of a single SD on BDNF expression, synaptic potentiation and protection against subsequent SD injury. **Methods:** KCl was used to induce SD in the hippocampal CA1 subregion of murine brain slices. Field excitatory postsynaptic potentials (fEPSPs) were recorded; q-PCR was used to measure BDNF mRNA expression. Theta-burst stimulation (TBS) was used to test long-term potentiation. In some experiments, slices were preconditioned with SD and later challenged with a second SD in metabolically compromised conditions. **Results:** In healthy brain slices, fEPSP slope was persistently increased by ~20%, 20 min after a single SD ($P<0.02$ vs. baseline, n=11). Both total and activity-dependent (L) BDNF increased by ~2-fold reaching peak levels at 45 minutes after SD ($P<0.01$ and $P=0.003$ for total and L, respectively; n=5-7). Potentiation after SD was unaffected by the A2A receptor antagonist, ZM241385. When tested at peak BDNF increases (i.e. 45 min after SD), TBS-induced potentiation was not enhanced, in comparison with SD naive time-matched control slices. We next evaluated if preconditioning improved recovery from SD in conditions of brain slice metabolic compromise. SD preconditioning improved fEPSP recovery to ~40% of baseline compared to only ~20% recovery in control slices ($P=0.03$, n=6-7). A combination of ketamine (30uM) pre-exposure with SD preconditioning further increased recovery to ~85% after SD (n=6). **Conclusion:** These data suggest BDNF is significantly upregulated after a single SD, although mechanisms other than adenosine A2 receptor activation likely couple any effect of BDNF to synaptic strengthening. Preconditioning with a single SD was shown to partially protect slices from SD-mediated injury during subsequent metabolic challenge. Thus, in addition to well established damaging effects of SD, these data suggest that a single SD could promote adaptive plasticity and protection in surviving peri-infarct tissues.

Disclosures: J. Weisend: None. **K.M. Reinhart:** None. **A.P. Carlson:** None. **C.W. Shuttleworth:** None.

Poster

214. Ischemic Stroke II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 214.04/F8

Topic: C.08. Ischemia

Support: JSPS KAKENHI Grant 18K07392
Smoking Research foundation

Title: GPR3 is upregulated in rodent mast cells immediately after brain ischemia and modulates degranulation

Authors: *S. TANAKA[1], Y. HAMAKAWA[1], Y. YANASE[2], M. YAMAMOTO[1], H. SHIRAKI[1], K. HARADA[1], I. HIDE[1], N. SAKAI[1];
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Abstract: G protein-coupled receptor 3 (GPR3), a member of the class A rhodopsin-type GPCR family, is highly expressed in various neurons and oocytes. GPR3 is unique in its ability to constitutively activate Gαs protein without the addition of ligands that elevates the basal level of intracellular cAMP. We have reported that the neuronal expression of GPR3 enhances neurite outgrowth and modulates neuronal survival; however, the physiological functions of GPR3 following brain ischemia have not been elucidated. Here we investigated whether GPR3 expression is modulated following brain ischemia. We found that GPR3 mRNA expression transiently increased in the ischemic hemisphere as early as 4 h after transient middle cerebral artery occlusion in Wistar rats and C57BL/6 mice. Contrary to our expectations, GPR3 promoter activity decreased in neurons in the ischemic hemisphere. Analysis of Percoll density gradient fractions from the ischemic brain homogenate indicated that the mast cells are the source of GPR3 in the ischemic brain. Reportedly, brain ischemia stimulates mast cell degranulation and alters the permeability of the blood-brain barrier, thereby modulating vasogenic edema and immune cell infiltration. Therefore, we further assessed GPR3 expression in the bone marrow-derived mast cells (BMMCs). Notably, the degranulation of BMMCs was significantly induced by IgE-mediated dinitrophenyl conjugated human serum albumin (DNP-HSA) or adenosine triphosphate (ATP). In addition, the degranulation of BMMCs was significantly inhibited by the upregulation of intracellular cAMP. Moreover, GPR3 mRNA was highly upregulated as early as 1-2 h and declined when BMMCs were stimulated by DNP-HSA or ATP. Finally, the effect of GPR3 expression on the degranulation of BMMCs was investigated. Compared with BMMCs from wild-type mice, BMMCs from GPR3 knockout mice showed significantly increased degranulation in response to various degranulation stimuli. This is the first study on the expression of GPR3 in the mast cells in response to various stimuli. Taken together, these results

suggest that GPR3 plays a role in inhibiting the degranulation of the mast cells and modulates inflammatory responses triggered by brain ischemia.

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Poster

214. Ischemic Stroke II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 214.05/F9

Topic: C.08. Ischemia

Support: The California Table Grape Commission
NIA (AG033720)
NINDS (NS094881)

Title: Grape extract enriched diet alters gut microbiome to enhance post-stroke recovery of white matter against ischemia

Authors: *C. BASTIAN, D. SCERBO, R. ZHANG, J. DAY, A. MILLER, S. BRUNET, S. BALTAN;
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Abstract: Stroke affects both gray (GM) and white matter (WM) of the brain. Previous studies suggest that grape-enriched diet protects GM against ischemia-reperfusion injury. Grape constituents have been described to alter gut microbiota, regulate the gut-brain axis and improve cognitive functions. It is not known whether a grape-enriched diet can preserve glial cells and protect WM integrity against stroke. Hence, we hypothesize that grape powder diet will promote axon function recovery following oxygen and glucose deprivation (OGD) by regulating intestinal microbiome and preserving axonal mitochondrial structure and motility.
Adult male C57BL6/J or Thy-1 mitoCFP mice (3-5 months of age) were fed 5% or 10% grape powder diet or respective control (added fructose and glucose) or normal (no added glucose or fructose) diets for up to 10 weeks. Mouse optic nerves (MONs), which are pure white matter tracts, were used to study axon function recovery by quantifying the area under evoked compound action potentials (CAPs) following OGD (60 min). Cryosectioned MONs were immunostained for glial markers. Mitochondrial live imaging studies were performed on MONs from Thy-1 mitoCFP mice. DNA extracted from fecal pellets were used for microbiome analysis and qPCR quantification.
Animals on grape powder diet (8-10 weeks) promoted oligodendrocyte survival and preserved axon function recovery when exposed to OGD. Grape powder diet at 5% or 10% equally protected and promoted axon function. In the grape powder diet group, axonal mitochondrial

structure and motility was preserved during and after OGD. There was no phylogenetic diversity of the gut microbiota between normal, control, and grape diets. Interestingly, phylum-level taxonomic profile showed that the control and grape diets led to an increase in *Firmicutes* compared to normal diet. Quantitative PCR analysis showed an increase in *Bacteriodetes* bacteria in the grape diet group compared to control.
We conclude that grape powder diet promotes white matter integrity against ischemic injury by modifying the gut microbiome. The bacterial metabolites that confer this gut-brain axis protection is currently being investigated.

Disclosures: **C. Bastian:** None. **D. Scerbo:** None. **R. Zhang:** None. **J. Day:** None. **A. Miller:** None. **S. Brunet:** None. **S. Baltan:** None.

Poster

214. Ischemic Stroke II

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Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 214.06/F10

Topic: C.08. Ischemia

Support: NRF-2018M3A9E8023851
NRF-2018R1C1B6006145

Title: Toll like receptor 2 mediated oligodendrocyte protective effect is exerted through p38 and NFkB

Authors: X. JIN[1], S. CHOWDHURY[1], ***J. CHOI**[2], B. KIM[2];
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Abstract: Oligodendrocyte (OL) is the myelinating glia of central nervous system. Myelin enhance signal conduction and maintain axonal integrity. Neurological deficits occurs when ischemic white matter damage causes OL loss and subsequent ischemic demyelination. Therefore, treating OL loss is an important therapeutic target for ischemic white matter damage. Previously, we found that toll like receptor 2 (TLR2) expression in OLs. TLR2 in OLs provide cell-autonomous protective effects on ischemic OL death. Here, we sought to identify the intracellular signaling pathways of TLR2, which are involved in TLR2 mediated OL protection. Treatment of Pam3CSK4 (Pam3), a well-known TLR2 agonist, induced phosphorylation of p38, ERK1/2, CREB, NFkB but not AKT. The same pathways were activated in response to Pam3 treatment following oxygen glucose deprivation (OGD). To identify a specific pathway causally linked to the TLR2 mediated OL protection, each pathway was inhibited pharmacologically or genetically using specific inhibitors or siRNA, respectively. The extent of OL death was measured using LDH assay. Among all tested, p38 MAPK inhibitor and NFkB inhibitor

significantly attenuated the TLR2 mediated OL protection. Knockdown experiments using siRNA confirmed functional implication of the two pathways in TLR2 mediated OL protection. Our results demonstrated that TLR2 provides OL protective effects under ischemic condition through p38 and NFkB pathways. Further studies will be needed to clarify more detailed signaling pathways that confer OL protection.

Disclosures: **X. Jin:** None. **S. Chowdhury:** None. **J. Choi:** None. **B. Kim:** None.

Poster

214. Ischemic Stroke II

Location: Hall A

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Program #/Poster #: 214.07/F11

Topic: C.08. Ischemia

Support: Hacettepe University Scientific Research Projects Coordination Unit, Project Number: THD-2017-14025
Hacettepe University Scientific Research Projects Coordination Unit, Project Number: TDK-2019-17652

Title: Cerebral peri-microvascular glycogen utilization has a role in endothelium-pericyte interaction in mice

Authors: *G. URUK[1], S. YILMAZ-OZCAN[1], B. DONMEZ-DEMIR[1], H. KARATAS-KURSUN[1], E. EREN-KOCAK[1,2], J. DURAN[4,5], J. GUINOVART[4,5,6], T. DALKARA[1,3], M. YEMISCI OZKAN[1,3];
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Abstract: Glycogen stored in peri-microvascular astrocyte processes may be the principal endogenous source of energy under energy-deprived conditions. Therefore, disrupted brain glycogen turnover may result in microvascular impairment. To test this hypothesis, we studied the effects of brain glycogen utilization on microvasculature using both transgenic mice in which glycogen synthase-1 is ablated via central nervous system (CNS) based cre-recombinase technology (Nestin-Cre) and/or glycogen phosphorylase inhibitor 1,4-Dideoxy-1,4-imino-D-arabinitol hydrochloride (DAB) injected mice. C57Bl/6 (25-30g, n=6), GYS-1 Nestin Knock-out (GYS-$1^{NestinKO}$) (18-20g, n=3) and $GYS1^{Heterozygous}$ mice (20-25g, n=3) were sacrificed at 8-12 weeks of age. Another group of mice (30-35g) received DAB intracerebroventricularly (icv) (0.75µL, 0.25M), and after 0.5, 1, 3, 6, 9, 24 hours (n=3 for each group) were sacrificed. Coronal

brain sections (50µm-thick) were labeled with Lycopersicon esculentum Lectin (LEL) for visualizing vessels, PDGFR-β, NG2 and CD13 for pericytes, Aquaporin-4 and GLUT-1 for astrocyte endfeet, Claudin-5 and zonula occludens-1 (ZO-1) for endothelial tight-junction assessment. Mean number of pericytes were quantified by a semi-stereological method (total online images of 10 3D-disectors (240x160x40µm) for each hemisphere/brain sections. We provide preliminary evidence that CNS peri-microvascular glycogen contributes to neurovascular integrity. One hour after DAB injection, PDGFR-β expressing pericytes decreased (180.12±3.06 pericytes/mm^2) when compared to icv. saline injected littermates (332.75±5.21 pericytes/mm^2) and started to detach (30.12% vs. 20.86% detached). At 6th hour of injection, most of the pericytes are significantly detached from their respective microvessel wall (43.03%). $GYS1^{NestinKO}$ mice have less PDGFR-β expressing pericytes compared to $GYS1^{Heterozygous}$ littermates, both transgenics had diminished number of PDGFR-β positive pericytes when compared to wild-type (WT) mice. On the contrary, the number of NG2 and CD13 expressing pericytes increased significantly. Transgenic and DAB injected mice expressed less claudin-5, but showed comparable levels of ZO-1 compared to WT mice. Moreover, astrocyte endfeet expressed increased Aquaporin-4 and decreased GLUT-1 levels reciprocally. These data suggest that brain peri-microvascular glycogen has a role in endothelium-pericyte interaction and thus blood brain barrier (BBB) function in mice.

Disclosures: G. Uruk: None. **S. Yilmaz-Ozcan:** None. **B. Donmez-Demir:** None. **H. Karatas-Kursun:** None. **E. Eren-Kocak:** None. **J. Duran:** None. **J. Guinovart:** None. **T. Dalkara:** None. **M. Yemisci Ozkan:** None.

Poster

214. Ischemic Stroke II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 214.08/F12

Topic: C.08. Ischemia

Support: JSPS Grants-in-Aid for Scientific Research (19K16504)

Title: Aquaporin 4 knockout in mice attenuates paravascular space closure and neuronal activity reduction after water intoxication

Authors: *T. ISHIKAWA[1], M. UNEKAWA[2], Y. TOMITA[2], J. NAKAHARA[2], M. YASUI[1]; [1]Pharmacol., [2]Neurol., Keio Univ., Tokyo, Japan

Abstract: Rapid intraperitoneal water injection causes acute hyponatremia that creates an osmotic gradient driving for water entry into the brain, leading to subsequent cerebral edema. Paravascular space has been suggested to participate in the fluid circulation in cerebral cortex, however, it has not been clarified whether it changes during the development of cerebral edema

by water intoxication. Here we have established an *in vivo* imaging method with a closed cranial window under isoflurane anesthesia to observe the paravascular space and astrocytes using CAG-GFP transgenic mice and intraperitoneal injection of sulforhodamine 101, respectively. We simultaneously monitored electro-corticogram (ECoG), cerebral blood flow (CBF), heart rate, and arterial blood pressure to examine physiological responses up to 40 min after the bolus injection of water. We first confirmed that both hyponatremia and cerebral edema indeed occurred after water injection. Water injection also induced reduction of CBF and ECoG power but did not change heart rate and blood pressure. In addition, cell body of astrocytes swelled and paravascular space got narrower. Aquaporin 4 (AQP4) is exclusively localized in the astrocyte end-feet where cover the paravascular space. We found that the ECoG power reduction and paravascular space closure were alleviated but the CBF reduction was not in the AQP4 knockout mice. These results implicate that the regulation of paravascular space may play roles in modulating brain water circulation and brain edema formation, which might be controlled by AQP4.

Disclosures: **T. Ishikawa:** None. **M. Unekawa:** None. **Y. Tomita:** None. **J. Nakahara:** None. **M. Yasui:** None.

Poster

214. Ischemic Stroke II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 214.09/F13

Topic: C.08. Ischemia

Support: American Heart Association Grant 17SDG33410777
NIH P01HL095070-06
FAPESP 2017/14020-4

Title: Poldip2 deficiency protects against leukocyte infiltration into the brain following ischemic stroke

Authors: L. EIDSON[1], A. CAMPOS[2], E. FAIDLEY[1], B. LASSÈGUE[1], C.-Y. KUAN[3], Y.-Y. SUN[3], M. G. TANSEY[1], K. GRIENDLING[1], ***M. S. HERNANDES**[1];
[1]Emory Univ., Atlanta, GA; [2]2Department of Neurosci., Hosp. Sírio-Libanês, Sao Paulo, Brazil; [3]Dept. of Neurosci., Univ. of Virginia, Charlottesville, VA

Abstract: Introduction: Poldip2 is a multifunctional protein that regulates the cytoskeleton, the extracellular matrix and alters matrix metalloproteinase activity. Poldip2 depletion has previously been reported to abrogate pro-inflammatory cytokine induction and blood-brain barrier disruption in stroke. Here we investigated Poldip2 as a novel regulator of leukocyte infiltration into the brain, following ischemic stroke. **Methods:** Transient cerebral hypoxia-

ischemia was induced in Poldip2$^{+/+}$ and Poldip2$^{+/-}$ mice. Brains were isolated 48 hours after cerebral ischemia, and half brain was immediately minced on in 1xHBSS and transferred to a papain and dispase II solution, incubated for 20 minutes at 37° C, and then neutralized with 10% FBS. Tissue was homogenized in cold 1xHBSS using a fine glass pipette. Homogenates were filtered through a 70um cell strainer, and then separated via percoll gradient centrifugation. Immune cells were collected and processed for multi-color flow cytometry to analyze peripheral immune cell infiltration. RT-PCR was used to measure mRNA levels of classical and alternative glial activation markers 24 hours after cerebral ischemia. **Results:** Quantitative flow cytometric analysis of CD45 expression, a leukocyte common antigen, demonstrates that CD45^{+} cells were significantly less abundant in the ischemic brains of Poldip2$^{+/-}$ mice when compared to Poldip2$^{+/+}$ mice ($p<0.01$). Further characterization of invading cell populations demonstrated that CD3^{+}, CD8^{+} and CD4^{+} T cells, CD11b^{+}Ly6G^{+} neutrophils and CD11b^{+}Ly6G^{-} dendritic cell counts were not significantly increased in the brain of either Poldip2$^{+/+}$ or Poldip2$^{+/-}$ mice 48 hours after stroke. However, the infiltration of CD45^{+}CD11b^{+} myeloid cells, CD45^{high}Ly6C^{high} and CD45^{high}MHCII^{low} inflammatory monocytes/macrophages was significantly reduced in Poldip2$^{+/-}$ mice after stroke when compared to Poldip2$^{+/+}$ mice ($p<0.0$, $p<0.05$, and $p<0.01$, respectively). Resistin-like molecule α (FIZZ1) and IL-1α were not significantly increased in the ischemic brains of either Poldip2$^{+/+}$ or Poldip2$^{+/-}$ mice. In Poldip2$^{+/+}$ ischemic brains, mannose receptor (MRC), Ym-1 and IL-1β mRNA expression were upregulated ($p<0.05$) 24 hours after ischemic stroke, but no induction of these mRNAs was observed in Poldip2$^{+/-}$ mice. However, baseline mRNA expression levels of Ym-1 and IL-4 were increased in the brains of Poldip2$^{+/-}$ mice when compared to Poldip2$^{+/+}$ mice ($p<0.05$). **Conclusions:** Poldip2 regulates leukocyte recruitment into ischemic tissue. Additionally, our data suggest that Poldip2 has profound effects on baseline and stroke-induced neuroinflammation.

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Poster

214. Ischemic Stroke II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 214.10/F14

Topic: C.08. Ischemia

Title: A1 astrocytes are neurotoxic after stroke

Authors: *T. C. PETERSON[1], A. E. MUNCH[2], E. BRAHMS[2], M. M. WEIGEL[2], K. INOUE[1], B. BARRES[2], M. BUCKWALTER[2], S. A. LIDDELOW[3];

[1]Univ. of North Carolina - Wilm, Wilmington, NC; [2]Stanford Univ., Stanford, CA; [3]New York Univ., New York, NY

Abstract: Microglia and astrocytes perform crucial central nervous system functions. Multiple different neurological disorders, like neurodegenerative disorders or neural insults such as stroke create a neuroinflammatory response that leads to glial cell activation. These cells change their conformation and take on multiple different phenotypes, causing them to lose much of their normal functions and take on an inflammatory role. Activation of microglia following injury leads to the conversion of astrocytes into an "A1" phenotype that is more neurotoxic in (Parkinson's Disease and optic nerve crush models). Three cytokines, tumor necrosis factor (TNF), interleukin 1a (Il-1a), and complement component 1q (C1q) are released from microglia and have been determined to induce the A1 astrocyte phenotype. We prevented "A1" neurotoxic astrocyte formation after stroke with triple knockout (TNF-, Il-1a-, and C1q-) mice, and compared their neuroinflammatory response and stroke size to wildtype mice. We hypothesized that inhibition of "A1" astrocytes in triple knockout mice would lead to less astrogliosis and reduced infarct size. Following distal middle cerebral artery occlusion, the infarct size of triple knockout mice was significantly less than wildtype mice at both 1 ($p < 0.05$) and 7 days ($p < 0.0001$) post-ischemia. We also found a reduction in astrogliosis in the triple knockout animals 7 days post-stroke ($p < 0.05$). No significant reduction of infarct size ($p = 0.4343$) was found 28 days post-dMCAO, but there was a reduction in astrogliosis ($p < 0.05$) in the triple knockout animals. Removing "A1" neurotoxic astrocytes from the neuroinflammatory response to stroke led to a reduction in the glial response to stroke and reduced infarct volume. Utilizing triple knockout animals to remove these A1 neurotoxic astrocytes has shown to be beneficial in multiple other animal models of neural injury and neurodegenerative disorders. Understanding the neurotoxic phenotype of this astrocyte activation and its feedback role on other resident and infiltrating cells could lead to treatments to reduce an exacerbated neuroinflammatory response. Alternative ways to inhibit A1 astrocytes should be investigated as possible treatment options for stroke and other neurodegenerative diseases.

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Poster

214. Ischemic Stroke II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 214.11/F15

Topic: C.08. Ischemia

Support: NIH Grant NS076620

Title: CSF1R antagonism abolishes ischemic preconditioning mediated protection in white matter

Authors: M. HAMNER, D. GONG, G. ROJAS, C. LEE, A. MCDONOUGH, B. R. RANSOM, ***J. R. WEINSTEIN**;
Neurol., Univ. Washington, Seattle, WA

Abstract: Ischemic preconditioning (IPC) is a robust protective phenomenon whereby brief ischemic exposure confers tolerance to a subsequent ischemic challenge. IPC has been studied primarily in gray matter predominant models where infarct volume was primary outcome. However, stroke frequently involves white matter (WM) and we reported that IPC mediated axonal protection occurs in the mouse optic nerve (MON), a pure WM tract. Protection was dependent on innate immune signaling pathways: TLR4 and type I interferon. Colony stimulating factor-1 (CSF1) signaling is critical for microglial survival and CSF1 receptor antagonism with pharmacologic agents including PLX5622 results in depletion of microglia in brain. Here we set out to determine if pretreatment with PLX5622: (i) depletes microglia in MON and (ii) influences IPC-mediated axonal protection. Following a 21 day treatment with either PLX5622-infused or vehicle-infused (control) chow, we delivered a brief *in vivo* preconditioning ischemic insult (unilateral common carotid artery ligation) to 12-16 week old mice and determined WM ischemic vulnerability (sensitivity to oxygen-glucose deprivation or OGD) 72 hours later, using acutely isolated MONs from the preconditioned (ipsilateral) and control (contralateral) hemispheres. Functional and structural recovery was assessed by quantitative measurement of compound action potentials (CAP) and immunofluorescent microscopy. PLX5622 treated mice showed marked depletion (>95%) of microglia in MON as determined by Iba1 and Tmem119 immunostaining. As expected, preconditioned MONs from control-chow treated mice showed better functional recovery after OGD than non-preconditioned MONs (39±3% vs. 24±2% normalized CAP area, N = 6, $p < 0.01$). In contrast, preconditioned MONs from PLX5622 treated mice had similar recovery to non-preconditioned MONs (27±2% vs. 30±2%, N = 6, p = NS). Immunohistochemical analyses of phosphorylated neurofilament demonstrated improved axonal integrity in the preconditioned control-, but not PLX5622-, treated animals, confirming the electrophysiologic results. These findings indicate that CSF1R antagonism abolishes IPC-mediated axonal protection in WM. This effect could be due to either disruption of CSF1R signaling and/or microglial depletion.

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Poster

214. Ischemic Stroke II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 214.12/F16

Topic: C.08. Ischemia

Support: NRF-2018R1A6A3A01013415
NRF-2018R1D1A1B07048239
NRF-2018R1D1A1B07050957
HI16C1012

Title: Beneficial effects of high-frequency repetitive transcranial magnetic stimulation in neuronal cell models of ischemia/reperfusion injury

Authors: *S. JO[1,2,3,4], A. BAEK[1,2,4,5], J. YU[1,2], J. SEO[1,2], Y.-K. SHIN[1,2], S. WI[1,2], S.-Y. SONG[1,2,6], B.-G. NAM[1,2,6], S. PYO[1,2,3,4], E. CHO[1,2,3,4], J. HEO[1,6,2], J. KIM[5], S. KIM[5], S.-R. CHO[1,2,4,6];
[2]Rehabil. Med., [3]Brain Korea 21 PLUS Project for Med. Sci., [4]Yonsei Stem Cell Ctr., [1]Yonsei Univ., Seoul, Korea, Republic of; [5]Rehabil. Med., Yonsei Univ., Wonju, Korea, Republic of; [6]Nano Sci. and Technol., Yonsei Univ., Seoul, Korea, Republic of

Abstract: Repetitive transcranial magnetic stimulation (rTMS) is a nonaggressive therapy that can be used to diagnose and treat various neurological disorders. It is well known that high-frequency (> 3 Hz) stimulation generally results in facilitation, while low-frequency (< 1 Hz) rTMS induces reduction of synaptic efficiency. However, the effects of stimulation in different frequencies also remain unclear. Therefore, current study examined the differential effects of repetitive magnetic stimulation (rMS) regarding on frequencies and the therapeutic mechanisms in neuronal cell models of ischemia/reperfusion (I/R) injury.
First, Mouse neuroblastoma cells, Neuro-2a(N2a), were randomly divided into three groups—sham, low-frequency (0.5 Hz) and high-frequency (10 Hz) groups—and were stimulated 10 minutes per a day for three days. As a result, cell proliferation of high-frequency group was increased *via* up-regulation of neurotrophic factors. Then, low-frequency and high-frequency groups were characterized by RNA-seq transcriptome analysis. Among several pathways, long-term potentiation pathway is a significant pathway which is enriched in high-frequency group. Furthermore, there were several factors induced in high-frequency group, like phosphorylation of cAMP-response element binding protein (CREB), brain-derived neurotrophic factor (BDNF) transcription *via* activation of calcium (Ca^{2+})-/calmodulin-dependent protein kinase II (CaMKII)-CREB pathway.
Next, N2a cells were differentiated with retinoic acid and established for the *in vitro* neuronal model of I/R injury under oxygen glucose deprivation/reoxgenation (OGD/R) condition for 3 hr. After OGD/R injury, the differentiated N2a cells were randomly divided into three groups—OGD/R+sham, OGD/R+low-frequency and high-frequency groups—and were stimulated with rMS 10 minites for a day. High-frequency of rMS stimulation increases cell proliferation through activation of extracellular-regulated kinases and Akt signaling pathway and inhibits apoptosis in OGD/R injured cells. Furthermore, high-frequency of rMS stimulation increases Ca^{2+}-CaMKII-CREB signaling pathway, further leading to alternation of BDNF expression and synaptic plasticity in OGD/R injured cells.
Taken together, these studies will provide a better understanding of the therapeutic mechanisms

of rTMS. These mechanisms may also be applicable in neural stem cells, patient-derived induced pluripotent stem cells and brain organoids for future studies.

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Poster

214. Ischemic Stroke II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 214.13/F17

Topic: C.08. Ischemia

Support: NIH grant NS096987
NIH grant NS100494
American Heart Association grant 17GRNT33410181

Title: Attenuation of mitochondrial Zn^{2+} accumulation during early reperfusion reduces ischemic neuronal injury

Authors: Y. V. MEDVEDEVA, H. Z. YIN, E. SHARMAN, A. BAZRAFKAN, G. TIAN, N. MAKI, Y. AKBARI, ***J. H. WEISS**;
Univ. of California Irvine, Irvine, CA

Abstract: Despite high morbidity of ischemic stroke, there are as of yet no neuroprotective interventions with efficacy in humans. Whereas most prior efforts targeted Ca^{2+}, recent findings have highlighted contributions of Zn^{2+}. *In vitro* studies by us and others support the hypothesis that mitochondria are important sites of injurious Zn^{2+} effects; after entering neurons, Zn^{2+} can enter mitochondria, triggering mitochondrial dysfunction (including reactive oxygen species production, depolarization, and swelling), and contributing to cell death. In hippocampal slices subjected to oxygen glucose deprivation (**OGD**), we found that neuronal Zn^{2+} accumulation during OGD and its entry into mitochondria appear to precede and contribute to the induction of acute neurodegeneration. In addition, there is Zn^{2+} accumulation in CA1 mitochondria in the hour **after** sublethal episodes of OGD that may contribute to their delayed dysfunction. Using this slice model, we have now examined the progression of changes over 4-5 h after a sublethal episode of OGD, and report several findings: **1:** 4-5 h after OGD, mitochondria in CA1 neurons are markedly swollen (assessed using confocal microscopy) and their potential is significantly decreased compared to mitochondria from control slices. **2:** Synaptic activity (assessed as frequency of spontaneous postsynaptic currents) initially decreases after sublethal OGD, prior to markedly increasing (reaching a maximum ~2.5 h after OGD withdrawal of 218 ± 85 min^{-1}, compared to 38 ± 6 in control), and then decreasing again. **3:** Blockade of the mitochondrial Ca^{2+}

uniporter (with ruthenium red, RR) or Zn^{2+} chelation (with TPEN), for ~15 min shortly after OGD withdrawal substantially attenuated all of these changes, and helped to preserve normal mitochondrial morphology and potential as well as neuronal synaptic activity after 4-5 h. Finally, in pilot test of principle studies using an *in vivo* rat asphyxial cardiac arrest model, we found ~8 min asphyxia to induce considerable degeneration of CA1 neurons 4 h later associated with strong Zn^{2+} accumulation within many damaged mitochondria (as assessed by Timm's sulfide silver staining to detect loci of reactive Zn^{2+} combined with EM ultrastructural examination). IV infusion of RR immediately upon restoration of blood flow substantially attenuated both the mitochondrial Zn^{2+} accumulation and the mitochondrial and neuronal damage, providing a precedent for the possible therapeutic utility of post ischemic treatments targeting mitochondrial Zn^{2+} accumulation *in vivo*.

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Poster

214. Ischemic Stroke II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 214.14/F18

Topic: C.08. Ischemia

Support: NIH grant NS073779
AHA grant 18POST34070061

Title: Prior exposure to recurrent hypoglycemia causes post-ischemic ER stress via increased free radical production in treated diabetic rats

Authors: *A. K. REHNI, S. CHO, K. R. DAVE;
Neurol., Univ. of Miami Sch. of Med., Miami, FL

Abstract: Cerebral ischemia is a serious complication of diabetes. Antidiabetic drugs induce recurrent hypoglycemia (RH). Previously, we showed that prior RH exposure enhances ischemic brain damage in insulin-treated diabetic (ITD) rats. However, the mechanism of this increase in ischemic injury is unknown. In the present study, we evaluated the hypothesis that enhanced acidosis causes an increase in free radical release, resulting in endoplasmic reticulum (ER) stress. Previously, we showed that the administration of an alkalizing agent (Tris-(hydroxymethyl)-aminomethane: THAM) decreases intra-ischemic acidosis in RH-exposed ITD (ITD + RH) rats. As acidosis increases the levels of free radicals, we determined that the administration of THAM decreases RH-induced post-ischemic increase in hydrogen peroxide production and microsomal calcium release in ITD + RH rats. Male Wistar rats were rendered diabetic by streptozotocin and, 2-3 weeks later, insulin pellets were implanted to treat hyperglycemia. After 1-2 weeks,

moderate hypoglycemia was elicited by dose(s) of insulin for 3 hours for 5 consecutive days. The following groups were employed: (A) ITD + vehicle (Veh) (n = 7): (B) ITD + RH + veh (n = 7) and, (C) ITD + RH + THAM (0.3 M, 3 ml / kg / hr, i.v.) (n = 7). Global cerebral ischemia (by bilateral carotid artery occlusion with hypotension) was induced for a period of eight minutes overnight after the last episode of hypoglycemia/equivalent time. THAM/Veh treatment was given 15 min before ischemia to 80 minutes of reperfusion. Hippocampii were harvested 23-25 h after ischemia. We measured the rate of hydrogen peroxide production in the homogenate using the Amplex Red fluorometric assay and microsomal calcium release. The rate of H_2O_2 production in ITD + RH rats 24 h after ischemia (128 ± 12 AFU/min/mg protein) was significantly higher (58%; $p<0.01$) than that of the ITD control group (81 ± 9 AFU/min/mg protein). The rate of H_2O_2 production in THAM-treated rats (81 ± 11 AFU/min/mg protein) was significantly lower (37%; $p<0.05$) than that of the RH + ITD + Veh control group. However, we did not observe any significant difference between ITD and ITD + RH + THAM groups. We are in the process of determining calcium release in microsomes. Our results, so far, demonstrate that ischemic acidosis increases the rate of H_2O_2 production in ITD + RH rats. Given the lack of understanding of the role played by hypoglycemia in mediating diabetic aggravation of ischemic brain injury, elucidating the mechanism of ischemic damage in RH-exposed ITD rats will help in identifying new therapeutic strategies to treat the serious condition in diabetics.

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Poster

215. Stroke and Ischemia I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 215.01/F19

Topic: C.08. Ischemia

Support: Vikings' Children Fund

Title: Proteomic analysis of synaptosomes revealed less inflammatory effect of perinatal ischemic stroke in TMEM35 (NACHO) knockout mice

Authors: *P. V. TRAN, A. BARKS, **M. BEESON**;
Dept. of Pediatrics, Univ. of Minnesota, Minneapolis, MN

Abstract: Background: Perinatal ischemic stroke (IPS) affects 1 in 5000 late preterm and full-term newborns and can cause neurodevelopmental impairments. Adult animal models of ischemic stroke implicate nicotinic acetylcholine receptors (nAChRs) in activation of inflammation. Whether this mechanism plays a role in IPS pathophysiology remains unknown. TMEM35 (NACHO) is required for cellular expression of nAchRs; thus, we probed the effects of *tmem35* knockout (KO) on synaptosomal proteins following IPS. Objective: Analyze synaptosomal proteins at 24-hr post-ischemia of postnatal day (P)10 WT and *tmem35*KO mice. Methods: P10 WT and *tmem35*KO littermates were anesthetized by inhalation isoflurane (3% induction, 2% maintenance) and randomized for right carotid artery ligation or not (Sham control). Following recovery from anesthesia, pups were reunited with nursing dams in home cages. At 24-hr post-ischemia, brains were bisected along the midline to separate ischemic (IL) and non-ischemic (CL) hemispheres. Synaptosomes were prepared using sucrose gradient and ultracentrifugation. Synaptosomal proteins were isolated and pooled from 2-3 samples for isobaric-tagged-for-absolute-and-relative-quantitation (iTRAQ 8-plexes) labeling and tandem LC-MS/MS. Following protein search and identification, differentially expressed proteins (DEPs) were analyzed by Ingenuity Pathway Analysis (IPA), a knowledge-based database. Results: 467 proteins were identified following exclusion criteria (Error factor < 2.0, peptide matches ≥ 3). Compared to WT CL hemisphere, 65 and 36 DEPs were identified from IL hemisphere of WT and KO, respectively. IPA mapped DEPs onto canonical pathways, indicating decreased synaptic LTP, CREB, alpha-adrenergic, and endothelin-1 signaling. However, KO mice uniquely showed decreased PI3K and IL-3 signaling. Conclusions: IPS alters expression of synaptosomal proteins suggesting reduced signaling pathways critical for neural plasticity. In *tmem35*KO pups, fewer DEPs were found, predicting less neuronal cell death and less impaired neuritogenesis. This neuroprotective effect was likely driven by lowering activity of the inflammatory pathways such as IL-3 signaling. Collectively, our findings implicate neural nAchRs in IPS pathophysiology.

Disclosures: P.V. Tran: None. **A. Barks:** None. **M. Beeson:** None.

Poster

215. Stroke and Ischemia I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 215.02/F20

Topic: C.08. Ischemia

Title: Immediate and delayed decrease of long term potentiation and memory deficits after neonatal intermittent hypoxia

Authors: I. GOUSSAKOV, S. SYNOWIEC, ***A. DROBYSHEVSKY**;
NorthShore Univ. HealthSystems, Evanston, IL

Abstract: Apnea of prematurity is a common clinical condition that occurs in premature infants and results in intermittent hypoxia (IH) to brain and other organs. While short episodes of apnea are considered of no clinical significance, prolonged apnea with bradycardia and large oxygen desaturation is associated with adverse neurological and cognitive outcome. The mechanisms of cognitive deficits in IH are poorly understood. We hypothesized that brief but multiple episodes of severe oxygen desaturation accompanied by bradycardia may affect early and late synaptic plasticity and produce long-term cognitive deficits. C57BL/6 mouse pups were exposed to IH paradigm consisting of alternating cycles of 5% oxygen for 2.5 min and room air for 5 to 10 min, 2 hours a day from P3 to P7. Long term potentiation (LTP) of synaptic strength in response to high frequency stimulation in hippocampal slices were examined 3 days and 6 weeks after IH. LTP was decreased in IH group relative to controls at both time points. That decrease was associated with deficits in spatial memory on Morris water maze and context fear conditioning test. Hypomyelination was observed in multiple gray and white matter areas on in vivo MRI using micromolecule proton fraction and ex vivo diffusion tensor imaging. No difference in caspase labeling was found between control and IH groups. We conclude that early changes in synaptic plasticity occurring during severe episodes of neonatal IH and persisting to adulthood may represent functional and structural substrate for long term cognitive deficits.

Disclosures: I. Goussakov: None. **S. Synowiec:** None. **A. Drobyshevsky:** None.

Poster

215. Stroke and Ischemia I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 215.03/F21

Topic: C.08. Ischemia

Title: CCR2CreER mice for fate mapping monocytes during brain development and after ischemic stroke

Authors: *H.-R. CHEN[1], Y.-Y. SUN[1], I. KUAN[2], C.-Y. KUAN[1];
[1]Univ. of Virginia Sch. of Med., Charlottesville, VA; [2]Emory Univ., Atlanta, GA

Abstract: The specific functions of monocyte-derivatives have been difficult to ascertain because monocytes quickly change their cell surface markers towards a microglia-like profile after entering the brains, making it difficult to be distinguished from the pre-existing microglia. Hence, there is a need for better genetic tools to identity monocyte-derivatives in fate-mapping studies. Using the BAC recombineering technology, we have obtained more than 10 founders and derived 3 F1 lines of CCR2-CreER mice. After crossed with R26R-GFP reporter mice and dosed by tamoxifen once daily IP for 5 days at P60, we compared the efficiency and specificity in peripheral blood, bone marrow, and spleen cells of monocyte-labeling via flow cytometry analysis in three transgenic CCR2-CreER lines. We then use the best line for further studies.

During normal development, Tamoxifen-induction at E14 labeled tangential migrating Iba1+ monocyte-derivatives in the meninges in P2 CCR2CreER bi-transgenic mice; while dosed by tamoxifen at E17 labeled ramified Tmem119+P2RY12+IbaI+ monocyte-derivatives in clusters located near the whisker-related barrel field of the somatosensory cortex and the neocortical pia at P24. In ischemic stroke injury model, we dosed by tamoxifen at P14-P15 followed by photothrombotic stroke injury at P16, we found a large number of proliferating GFP+ monocyte-derivatives surrounding the infarct region as a ring structure at 72 h post P16 stroke. Further, these ameboid-shape monocyte-derivatives not only express Iba1and the blurry/low levels of Tmem119 and P2RY12, but also CD68 and TNFain the ipsilateral hemisphere, suggesting that they are in a pro-inflammatory state. In long-term fate-mapping monocyte-derivatives at 30 days post P16 stroke, those GFP+ monocyte-derivatives not only express evaluated Tmem119 and P2RY12, but also Iba1, CD68 and TNFa, suggesting that they are in a chronic inflammatory state. Together, we anticipate the CCR2CreER mice will be value for the future study of monocyte-derivatives in normal development and disease models.

Disclosures: H. Chen: None. **Y. Sun:** None. **I. Kuan:** None. **C. Kuan:** None.

Poster

215. Stroke and Ischemia I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 215.04/F22

Topic: C.08. Ischemia

Support: NIH Grant DA029121

Title: Maternal electronic cigarette use can cause cognitive dysfunction in offspring & increased sensitivity to hypoxic-ischemic brain injury

Authors: *A. E. SIFAT, S. NOZOHOURI, H. VILLALBA, B. VAIDYA, T. ABBRUSCATO; Pharmaceut. Sci., Texas Tech. Univ. Hlth. Sci. Ctr., Amarillo, TX

Abstract: Prenatal exposure to tobacco smoke and nicotine is believed to interfere with fetal brain development predisposing offspring to different neurobehavioral and neuropsychological disorders. Included in this is increased neonatal vulnerability to hypoxic-ischemic encephalopathy (HIE) which is a major cause of neonatal death and child disability in the US. These effects could be, in part, mediated by fetal nicotine exposure. Use of electronic cigarettes (e-Cigs), commonly known as vaping, has rapidly increased in recent times in the general population, including women of reproductive age. E-Cig use during pregnancy is also increasing because of the addictive properties of nicotine along with the perception of the relative safety of e-Cigs. In this study, we aimed to investigate the effects of maternal e-Cig use on neonatal brain development and HIE utilizing a combination of in vitro and in vivo models. Pregnant CD1 mice

were exposed to e-Cig vapor (2.4% nicotine). Primary cortical neurons were isolated from e-Cig exposed fetus and cultured for seven days with subsequent exposure to oxygen-glucose deprivation followed by reoxygenation (OGD/R) to mimic ischemia-reperfusion injury. Hypoxic-ischemic brain injury was induced in 8-9 days old mouse pups by a combination of left common carotid artery ligation and 15 minutes exposure to 8% oxygen. We found that e-Cig exposed cortical neurons demonstrated decreased cell viability and increased cleaved poly ADP-ribose polymerase 1 (PARP1) expression in OGD/R conditions. These effects were accompanied by decreased glucose uptake & glucose transporter expression, and decreased ATP content in OGD/R condition. Our preliminary data also indicate increased sensitivity to HI brain injury in prenatally e-Cig exposed mouse pups. Additionally, in utero e-Cig exposed mice offspring displayed a significantly increased level of hyperactivity and impaired memory at postnatal day 45 in open field and novel object recognition test respectively. These results indicate that maternal e-Cig exposure could lead to offspring behavioral abnormalities and enhance HI brain injury. Further studies are needed to demonstrate the long-term anatomical and behavioral effects of HI brain injury in e-Cig exposed offspring. This study is instrumental in elucidating the possible deleterious effects of maternal e-Cig use in the general population.

Disclosures: **A.E. Sifat:** None. **S. Nozohouri:** None. **H. Villalba:** None. **B. Vaidya:** None. **T. Abbruscato:** None.

Poster

215. Stroke and Ischemia I

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Program #/Poster #: 215.05/F23

Topic: C.08. Ischemia

Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq-Brazil)
Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES-Brazil)
Pró-Reitoria de Pesquisa (PROPESQ/UFRGS-Brazil)
Fundo de Incentivo à Pesquisa e Eventos do Hospital de Clínicas de Porto Alegre (FIPE/HCPA-Brazil)

Title: Effects of lactate on the brain following neonatal hypoxia-ischemia: Preliminary findings

Authors: *I. D. TASSINARI[1], T. L. RODRIGUES[1], M. K. G. ANDRADE[1], D. N. MACHADO[1], R. B. FABRES[1], E. B. M. ARAUJO[1], R. R. NUNES[1], A. H. PAZ[2], L. S. DE FRAGA[1];
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Abstract: Besides being a metabolic substrate, lactate (LAC) is now receiving more attention due to its putative neuroprotective effects on the brain. Neonatal hypoxia-ischemia (HI) is one of the major causes of neurologic disabilities in newborns. Moreover, LAC administration has been shown to be neuroprotective in models of cerebral ischemia in adult animals. Here, we evaluated the effects of LAC in a neonatal model of HI. Seven-days-old male Wistar rats (n=4) underwent a surgery for common right carotid artery ligation followed by exposure to a hypoxic atmosphere (8% of oxygen) for 60 min at 37ºC. Animals were assigned to four experimental groups: HI (rats submitted to HI), HILac (rats submitted to HI that received LAC), SHAM (rats underwent fictitious surgery) and SHAMLac (SHAM rats that received LAC). LAC was administered intraperitoneally after HI twice a day for 5 consecutive days. Behavioral tasks such as righting reflex and negative geotaxis were evaluated at P8, P14 and P20. Two weeks after HI (P21) animals were euthanized and their brains were processed for hematoxylin-eosin staining in order to evaluate the volume of brain lesion. Data were analyzed by ANOVA followed by Bonferroni. Latency to righting (righting reflex) was similar among groups ($p>0.05$). However, animals from HI group took longer to turn 180º in the negative geotaxis task at P8 ($p<0.05$). This neurological impairment was reverted in animals from HILac group. On the other hand, LAC was not able to reduce lesion volume ($p>0.05$). In summary, even without reducing brain damage, LAC administration ameliorated the neurological reflex suggesting a neuroprotective role of lactate. These are initial results and additional experiments are required to confirm this interesting outcome of LAC administration following HI insult.

Disclosures: I.D. Tassinari: None. **T.L. Rodrigues:** None. **M.K.G. Andrade:** None. **D.N. Machado:** None. **R.B. Fabres:** None. **E.B.M. Araujo:** None. **R.R. Nunes:** None. **A.H. Paz:** None. **L.S. de Fraga:** None.

Poster

215. Stroke and Ischemia I

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Program #/Poster #: 215.06/F24

Topic: C.08. Ischemia

Support: NIH Grant R15 HD077544-03

Title: Effects of early anti-inflammatory treatment and timing of working memory training on long-term cognitive outcomes in a model of neonatal hypoxia-ischemia

Authors: *A. B. BRADFORD[1], M. HERNANDEZ[1], E. KEARNEY[1], L. THEREALT[1], Y.-P. LIM[2,3], B. S. STONESTREET[4], S. THRELKELD[1];
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Abstract: Hypoxic-Ischemic (HI) brain injury, common during human birth and the perinatal period, continues to be a significant problem causing life-long cognitive impairments. Early diagnosis may improve strategies for therapeutic intervention including those targeting the prolonged inflammatory cascade and/or emphasizing cognitive strategies later in life. In humans, optimal ages of intervention and effects on brain structure are difficult to study. A standard model of HI is used in rats to simulate injury at comparative developmental times to human birth. In this model, carotid ligation is carried out at P7, followed by hypoxia for 2 hours. We previously reported two intervention approaches that improve cognitive and sensory function in this rat model of HI injury. Firstly, Inter-alpha inhibitor proteins (IAIPs), a family of ubiquitous and structurally-related plasma proteins that modulate inflammatory responses to infection, improve cognitive and sensory processing when administered during early HI injury. Secondly, early training regimens, using an eight-arm radial water maze (RWM) task, improved successive cognitive performance in comparison to shams and HI subjects with no early training. Here we expanded these studies to assess the combined effects of early IAIPs treatment and RWM training, at two developmental time points, in order to identify possible critical windows for behavioral intervention. Sixty-seven male Wistar rats were divided into three groups; HI injury (n=20 post-surgery), sham surgery (n=22), and HI injury with two perinatal IAIPs injections (n=25). In order to assess cognitive intervention timing, subsets of the three treatment groups received either “early” RWM followed by a retest session (P31-P50 and P74-P92) or “late” RWM testing followed by a retest session (P53-P71 and P95-113). Together, results support our previous findings that combining behavioral and anti-inflammatory interventions in the presence of HI injury improve subsequent learning performance, and further indicate critical periods for behavioral intervention to improve cognitive outcomes on the RWM. Results from this study provide insight into normal brain development and the impact of developmentally targeted task-specific enrichment on subsequent cognitive processing, in addition to having significant implications for combinatorial anti-inflammatory and behavioral intervention following neonatal brain injury.

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Poster

215. Stroke and Ischemia I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 215.07/F25

Topic: C.08. Ischemia

Support: CNPq
FAPERJ

CAPES

Title: Long-term characterization of the gliofibrotic scar in a model of neonatal hypoxic-ischemic brain injury

Authors: *L. BOLINI, D. P. DANTAS, R. MENDEZ-OTERO, A. M. VALE, P. M. PIMENTEL-COELHO;
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Abstract: Hypoxic-ischemic encephalopathy (HIE) is an important cause of death amongst children, corresponding to 11% of the deaths of children up to 5 years old in developing countries. HIE children can be treated with therapeutic hypothermia within 6 hours after birth, but early death or survival with long-term disabilities/impairments are still possible outcomes. In adults, neuronal death is usually followed by scar formation on the injured tissue, which usually has a fibrotic component. In this regard, astrocytes, pericytes and microglia, as well as peripheral myeloid cells, have been shown to have crucial roles on the formation of the glial scar and on the repair of the adult central nervous system. In this study, we characterized the formation of the glial scar in a murine model of HIE. Post-natal day 10 Balb/c mice were subjected to the Rice-Vannucci model of HIE: permanent ligation of the right common carotid artery followed by 80 min in a hypoxic chamber at 8% O_2. Animals were deeply anesthetized and then transcardially perfused with ice-cold 0,9% saline solution, followed by 4% paraformaldehyde, at different time points post-injury. Immunofluorescence analysis of coronal brain slices with confocal microscopy showed extracellular matrix remodeling with deposition of proteins such as fibronectin and laminin, from as early as 7 days to up to 7 weeks after the injury, in the ipsilateral cerebral cortex. This occurred in association with the formation of a fibroblastic reticular network (expressing ER-TR7 antigen) at 4-7 weeks post-injury, as well as with an increase in the number of astrocytes ($GFAP^+$ cells) and microglia/macrophages ($F4/80^+$ cells) from 3 days for up to 7 weeks after the injury. We also noticed histomorphological differences in the composition of the glial scar when the hippocampus and the striatum were compared to the cerebral cortex. The striatal and hippocampal scars were mainly glial, with less fibrotic components. Moreover, microglia/macrophages were found both in the core and the periphery of the cortical scar, whereas astrocytes were mainly found in the border of the scar. In contrast, in the hippocampal and striatal scars microglia/macrophages and astrocytes were intermingled. Finally, we observed the persistent presence of serum proteins, such as albumin and fibrinogen, in the glial scars. In conclusion, these data suggest that HIE induces the formation of glial scars with distinct characteristics in different brain regions.

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Poster

215. Stroke and Ischemia I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 215.08/F26

Topic: C.08. Ischemia

Title: An innovative neuro-protective role for galantamine in a neonatal mouse model of white matter injury

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Abstract: Background: WMI is the most common cause of cerebral palsy in premature infants. Microglia is the main inflammatory cell involved in its pathogenesis. We propose that galantamine administration ameliorates the inflammatory process leading to decreased WMI.
Objective: To elucidate the neuroprotective mechanism by which galantamine reduces hypoxic ischemic (HI) brain injury in neonatal mice.
Design/Methods: WMI neonate mouse model was established by temporary bilateral carotid ligation at P5, followed by hypoxia exposure 8% for 20 min. 4 neonate groups were studied. RA + saline , RA + Galantamine, (HI) + saline and (HI) + Galantamine. 2 doses of galantamine (5mg/kg dose) were injected IP at P5 (2 hours after HI insult) and P6. Choline acetyltransferase (ChAT) and acetylcholinesterase activity measured galantamine cholinergic activity. Anti-inflammatory effects of galantamine was studied on neurons and glia by immunofluorescence staining (NeuN, GFAP, Iba1, CD68, arginase 1, CNPase). Multiplex ELISA for Pro-inflammatory markers, western blot for phosphokinases (pP38, pERK, pAKT and pP65) , caspase 3 and reactive oxygen species assays (ROS) were performed.
Results: Galantamine treated pups showed minimal paresis and co-ordination deficits as compared to saline treated HI group, which had severe insult. Histopathology showed ventriculomegally, neuronal necrosis and apoptosis in saline treated HI group which was ameliorated with galantamine treatment. Galantamine has significant anti-inflammatory properties evidenced by a significant increase in ChAT expression and a decrease in acetylcholinesterase activity as well as a significant reduction in brain pro-inflammatory cytokines IL 4, IL6 and TNF α, microgliosis, and astrogliosis in galantamine treated HI vs saline treated HI. There was a shift towards an M2 microglial phenotype (increased arginase 1 and decreased in CD68), along with a significant decrease in HIF 1α, pP38, pERK, pAKT and pP65 in the HI galantamine treated vs. HI saline treated pups. A significant reduction in ROS levels contributed to improved myelination by preserving pre-oligodendrocytes in the HI galantamine versus HI saline.
Conclusion(s): Galantamine offers significant protection in WMI. Galantamine has a potent anti-inflammatory and antioxidant properties through the potentiation of the cholinergic activity

and the reduction of brain cytokine levels, NF-kB activation, microglial activation and ROS levels while preserving pre-oligodendrocytes and thus improving myelination in WMI.

Disclosures: **N. Zaghloul:** None. **K.R. Ayasolla:** None. **M.N. Ahmed:** None.

Poster

215. Stroke and Ischemia I

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Program #/Poster #: 215.09/F27

Topic: C.08. Ischemia

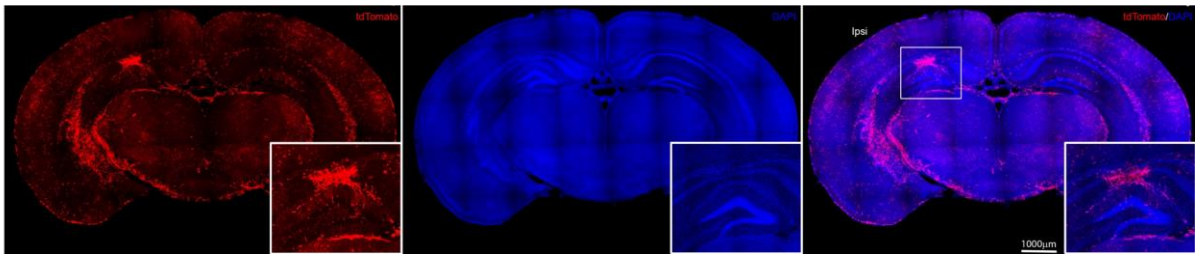
Support: NIH K08NS101122
NIH R01NS040337
NIH R01NS044370

Title: Persistent astrocyte activity in young adult mice following neonatal hypoxia-ischemia

Authors: D. SKWARZYNSKA[1], P. WAGLEY[1], M. MAZURKIEWICZ[1], N. EPLER[2], B. PACE[1], J. KAPUR[3], *J. BURNSED[1];
[1]Univ. of Virginia, Charlottesville, VA; [2]Univ. of Michigan, Ann Arbor, MI; [3]Dept Neurol., Univ. Virginia Hlth. Sci. Ctr., Charlottesvle, VA

Abstract: Background: Hypoxic-ischemic encephalopathy (HIE) affects 1.5/1000 newborns and is a major cause of neonatal seizures. Early-life seizures result in permanent changes to neuronal circuitry which likely have ramification on cognitive and memory outcomes. The goal of the study is to map neuronal circuits affected by hypoxia-ischemia (HI)-induced acute seizures and subsequent baseline neuronal activity in young adult mice following neonatal HI. **Methods:** HI was created using unilateral carotid ligation+45 min of 8% FiO2 in postnatal day (p)10 Cre-tamoxifen transgenic mice (TRAP). To tag active cells during seizures, mice were injected with 4-hydroxytamoxifen (4OHT) 30 min post-HI to allow expression of fluorescent protein (tdTomato) in active cells. HI or Sham (neck incision/equivalent exposure to anesthesia) mice were perfused and 200µm thick tissue was processed using tissue clearing methods. To examine baseline cell activation in young adult mice following neonatal injury, a separate group of TRAP mice were exposed to HI or Sham on p10, then injected with 4OHT on p30. Cleared tissue was immunostained for DAPI or GFAP, imaged on a confocal microscope, and analyzed using Imaris 9.1. **Results:** 100% of mice which underwent HI exhibit seizures during the time of hypoxia. Neuronal circuits affected by HI-induced acute seizures involve pathways crucial for memory acquisition and consolidation, such as hippocampal-parahippocampal circuit. *C-fos* expressing astrocytes were observed during acute HI seizures, most prominently in CA1. On p30, we observed clusters of *c-fos* expressing astrocytes most prominent in the area of neuronal loss in CA1, weeks after the initial injury (figure). **Conclusions:** Acute HI seizures result in abnormal

neuronal activity in circuits crucial for memory. There are clusters of persistently activate astrocytes remote from injury in the area of neuronal loss in CA1, which may have ramifications on memory function by disruption of memory circuitry. Ongoing work focuses on the role of these *c-fos* expressing astrocytes, remote from HI injury, play in memory defects.



Disclosures: D. Skwarzynska: None. **P. Wagley:** None. **J. Kapur:** None. **J. Burnsed:** None.

Poster

215. Stroke and Ischemia I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 215.10/F28

Topic: C.08. Ischemia

Support: NINDS R01NS086945

Title: Intrauterine growth restriction and hyperoxia downregulates myelin gene expression

Authors: J. CHANG[1], M. H. BASHIR[1], *R. W. DETTMAN[2], M. L. V. DIZON[3]; [1]Pediatrics, Northwestern Univ., Chicago, IL; [2]Anne and Robert H Lurie Children's Hosp., Chicago, IL; [3]Pediatrics, Northwestern University/Prentice Women's Hosp., Chicago, IL

Abstract: BACKGROUND: Intrauterine growth restriction (IUGR) is defined as a significant reduction in fetal growth rate resulting in birth weight <10th percentile for gestational age. It affects approximately 5% of pregnancies worldwide and results in increased mortality and morbidity (Tolcos 2010). IUGR infants have a 5-7 fold increase risk of developing cerebral palsy (CP) (Jacobbson 2008). It is unclear why certain IUGR infants go on to develop worse motor dysfunction and CP. Previously we've shown evidence of impaired white matter (WM) development in a murine model of IUGR in combination with postnatal exposure to hyperoxia (Chang 2018). We hypothesize that hyperoxia exposure acts as an additional risk factor to already high risk IUGR infants by specifically effecting WM during myelination. **OBJECTIVE:** To determine if IUGR with postnatal hyperoxia exposure results in differential expression of genes involved in myelination **DESIGN/METHODS:** Pregnant C57Bl6 mice were implanted with micro-osmotic pumps containing a thromboxane A_2 analog (U-46619) or 0.5% EtOH at E12.5. After spontaneous birth pups weighing <10th percentile at birth were categorized as IUGR. IUGR pups were placed into 75% FiO_2 from birth to P14. Brains were removed at P14

and total RNA was isolated using MirVana miRNA Isolation Kit. RNA libraries were constructed and sequenced by Northwestern University Sequencing Core Facility. Differential expression (DE) was determined using DESeq2, FDR-adjusted p-value < 0.05 cutoff for determining significance. Data was also analyzed with Ingenuity Pathway Analysis software. Validation was performed by TaqMan Advanced miRNA cDNA Synthesis Kit and real-time qPCR. **RESULTS:** There was significant downregulation of genes involved myelination including MoBP, PLP1, Mog, and CNP in IUGR/hyperoxia compared to vehicle ($p<0.05$, $n=6$). There was also significant downregulation of 34 genes specifically associated with mature myelinating oligodendrocytes (OLs) and 24 genes associated with newly-formed and myelinating OLs. Pathways analysis showed a significant activation of molecules specific to motor dysfunction/ movement disorders with z-scores >3. **CONCLUSIONS:** IUGR combined with postnatal hyperoxia results in downregulation of critical genes involved in myelination. Our results highlight a specific clinical risk factor to IUGR infants that results in WM injury and motor dysfunction. Our data also provides insight into changes that occur in the transcriptome and allows for future studies into targeted interventions.

Disclosures: J. Chang: None. **M.H. Bashir:** None. **R.W. Dettman:** None. **M.L.V. Dizon:** None.

Poster

215. Stroke and Ischemia I

Location: Hall A

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Program #/Poster #: 215.11/F29

Topic: C.08. Ischemia

Support: ISSSTE (RPI-223.2017)
CONACYT (708267)

Title: Cerebrolysin treatment improves motor abilities in neonatal rats with hypoxic ischemic encephalopathy

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Abstract: Hypoxic-isquemic encephalopathy (HIE) in neonatal period generates deficits in fine motor skills and muscle tone, cognitive and neuronal damage. Some brain structures affected by HIE are: cortex, hippocampus, putamen, ventrolateral thalami and dorsal mesencephalon. Neonatal brain is active in process like neurogenesis, gliogenesis, synapse formation and

mielination. For this reason, during this period of life brain maintains a large capacity for generating neurons after injury. Cerebrolysin is a mixture of peptides which mimicks actions of neurotrophic factors, its use has shown beneficial effects in neurodegenerative diseases through activation of mechanism of neuroprotection and repair, enhanced neurogenesis, proliferation and neural survival. We tested the hypothesis that administration of Cerebrolysin at P8-P14 might attenuate neuronal damage in neonatal rats with HIE reflected in motor abilities. Cerebrolysin treatment improve performance of experimental subjects with HIE in front-limb suspension test but, in other motor skill test like surface righthing, ambulation test and hindlimb foot angle did not observed differences. In brain structure, we did not observed presence of edema or other ultraestructural damage in association with Cerebrolysin administration. In addition, we analyze neurogenesis process in hippocampus and cerebral cortex induced by administration of Cerebrolysin related to improve motor habilities in neonatal rats with HIE through inmunoreactivity to BrdU (proliferative cell marker) and Neu-N (neuronal marker). Our results supports that Cerebrolysin treatment contribute to neurogenesis and attenuate neuronal damage, process that are associated with improve motor skills in rats with HIE.

Disclosures: E. Barrientos Zavalza: None. **A. Jimenez-Anguiano:** None. **B. Leon Chavez:** None. **J.A. Gonzalez-Barrios:** None.

Poster

215. Stroke and Ischemia I

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Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH R01 NS088192
NIH R21 NS107897
NIH R56 NS105632
Dr. Ralph and Marian Falk Medical Research Trust-Catalyst Award
CleaveBioscience Research Fund

Title: Repurposing cancer therapeutics to treatment of Huntington's disease via inhibition of VCP

Authors: *Y. SHANG, R. WANG, X. SUN, X. QI;
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Abstract: Huntington’s disease (HD) is an inherited and fatal neurodegenerative disorder with no treatment available. We have previously identified valosin-containing protein (VCP) as an interactor of mutant huntingtin (mtHtt), the disease mutant that causes HD. Recruited by mtHtt, VCP aberrantly translocates to mitochondria. The accumulation of VCP on mitochondria results

in mitochondrial depolarization, excessive mitochondrial fragmentation and aberrant mitophagy. These manifestations have been widely acknowledged to be crucial steps in initiation of neuronal degeneration in HD. Notably, abolishing VCP mitochondrial accumulation by blocking VCP/mtHtt interaction via a peptide inhibitor HV-3 reduced mitochondrial functional failure and subsequent neuropathology in HD models. These, together, make VCP a promising target for innovative therapy of HD. In the present study, we treat HD striatal cells with CB-5083 which is a selective VCP inhibitor that was used as an anti-cancer drug in clinical trial. We have found that, with a low dose of 200nM, CB-5083 significantly increases cell viability (40.2% ± 16.8) and mitochondrial membrane potential (MMP) (27.1% ± 4.18) in HD striatal cells. Moreover, treatment with CB5083 corrects excessive mitochondrial fragmentation (81.2% ± 10.77) and mitophagy (36.3% ± 4.80). With the same dose, CB-5083 has also shown neuroprotective effects in neurons derived from HD patient-induced pluripotent stem cells (HD iPSCs); the treatment improves MMP (216.76% ± 157.90), and suppresses neuronal cell death (31.8% ± 5.78) upon withdrawal of growth factor. The treatment also reduces dendrite (142.64% ± 38.26) and axon (212.61% ± 42.46) shortening of medium spiny neurons derived from HD iPSCs . The efficacy of CB5083 has further been tested in YAC128 transgenic mice which expresses full-length human mtHtt. Sustained treatment with CB-5083 improves general movement activity of YAC128 mice and reduces neuropathology associated with HD. In summary, our findings provide multiple lines of evidence *in vitro* and *in vivo* that targeting VCP might be a useful approach to reduce HD pathology and that CB-5083, an inhibitor of VCP, may be useful for development of HD therapy.

Disclosures: **Y. Shang:** None. **R. Wang:** None. **X. Sun:** None. **X. Qi:** None.

Poster

215. Stroke and Ischemia I

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Topic: C.08. Ischemia

Support: PRODEP NPTC UATLX-PTC-128
MGJ, & CONACyT CB-2015-255936 to OGF
CONACyT, OMN 930329

Title: Acute global cerebral ischemia decreases the population of pyramidal cells in the CA1 region of the hippocampus in female rats with 15 and 30 days of ovariectomy

Authors: *O. MONTES-NARVAEZ[1], O. GONZALEZ-FLORES[2], G. MORALI[3], M. GARCIA-JUÁREZ, SR[4];
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Abstract: Acute global cerebral ischemia decreases the population of pyramidal cells in the Ammon horn of the hippocampus. However, the animal models used for the study of this neuropathology have been mostly young and intact male rats, which are excellent for the description of molecular mechanisms but do not reflect the comorbidity of the most susceptible human population, which are the postmenopausal women. Thus, the establishment of menopause in the woman changes her physiology and the sensibility of her organism to benefices of replacement hormonal therapy, under this condition it has been pointed out that there is an optimal window period for the initiation of hormone replacement therapy treatments in postmenopausal women; however, this period not have been identified totally, and the neuroprotective treatments have been inefficient or adverse. Thus, in the present study, we studied the hypothesis that there is a differential effect on the time of absence of steroid hormones after ovariectomy for the decrease in the number of pyramidal cells in the CA1, CA2 and CA3 regions of the hippocampus, after an episode of acute global cerebral ischemia. For this, we used female rats of the Sprague Dawley strain with 15 or 30 days post-ovariectomy. They underwent the procedure of acute global cerebral ischemia according to the methodology proposed by Pulsinelli & Brierley through the occlusion of the 4 cerebral arteries. Once the procedure was completed, at 7 days post-ischemia, the animals were perfused with 10% formaldehyde, & the brain was extracted. Coronal sections of 16 μm were made at the level of the hippocampal formation and stained with cresyl violet. The results show that there is a considerable decrease in the number of pyramidal cells of the CA1 region, but not in the CA2 & CA3 regions, in both groups of female rats with 15 and 30 days of ovariectomy. These data show that both groups of rats show the same sensitivity to damage produced by global cerebral ischemia; however, we do not know if there will be the same response to neuroprotection by progesterone, which will be the objective for the following experiment.

Disclosures: O. Montes-Narvaez: None. **O. Gonzalez-Flores:** None. **G. Morali:** None. **M. Garcia-Juárez:** None.

Poster

215. Stroke and Ischemia I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 215.14/F32

Topic: C.08. Ischemia

Support: German Research Foundation (DFG, LI2534/2-1) to A.L.
European Research Council (ERC-StG 802305) to A.L.
Munich Cluster for Systems Neurology (EXC 2145 SyNergy) to A.L.

Title: The role of T cells in regeneration after stroke

Authors: *S. HEINDL, B. GESIERICH, N. FRANZMEIER, J. CRAMER, M. DUERING, A. LIESZ;
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Abstract: Stroke is among the leading causes of death and long-term disability worldwide.After stroke, complex neuroinflammatory mechanisms cause secondary brain damage, with T cells as key modulators. While unequivocal evidence for a pathomechanistic role of neuroinflammation has been shown for the acute phase after stroke, the impact of inflammatory cascades on chronic recovery after stroke is barely investigated. Despite this lack of information, clinical trials studying the effect of inhibiting T cell infiltration into the brain have been initiated but could not demonstrate improvement of clinical stroke outcome (clincalTrials.govNCT02730455). Thus, the complex role of immune cell infiltration and its impact on the outcome after stroke remain to be elucidated. Here, we aimed to investigate the role of immune cell infiltration - especially of T cells - on post stroke regeneration. The study was conducted in male and female mice. Permanent ischemia was induced by photothrombosis (PT). We analysed cerebral leukocyte infiltration by flow cytometry up to 3 months post-stroke. To examine T cell infiltration effects on post stroke regeneration, we repetitively injected mice with a monoclonal antibody against integrin alpha4 (aCD49d) over the post-stroke period. Functional recovery was assessed by behavior tests for focal deficits and *in vivo*widefield calcium imaging for cortical connectivity. Synaptic plasticity was evaluated peri-lesional using Golgi-Cox staining and immunohistology of excitatory synapses. Flow cytometry revealed leukocyte infiltration to the ischemic brain, which even more increased particularly in the T cell population during 6 months post stroke. aCD49d treatment reduced cerebral T_{Helper}cell counts at 1 month after stroke, whereas other lymphocyte populations were unaffected and the delayed increase in T cell invasion at chronic time points remained unaltered. Correspondingly, aCD49d treatment did not significantly alter behavioral and cortical network recovery. Moreover, we did not detect significant changes in histological markers of synaptic plasticity between the treatment groups. This study reveals that—unlike protective effects on acute stroke outcome—blocking cerebral leukocyte invasion by aCD49d treatment did not improve post stroke recovery. Our results indicate that this might be due to insufficient efficacy in preventing delayed cerebral T cell invasion and therefore did not modulate behavioral, imaging and histological recovery markers after stroke. These results are important in light of previously failed and currently performed clinical trials testing similar therapeutic approaches in stroke patients.

Disclosures: S. Heindl: None. **A. Liesz:** None.

Poster

215. Stroke and Ischemia I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 215.15/F33

Topic: C.08. Ischemia

Support: R01HL071568

Title: Cerebral perfusion and cerebrovascular autoregulation after resuscitation from asphyxia cardiac arrest rat model

Authors: *Q. WANG[1], Y. ZENG[1], H. MODI[1], J. SENARATHNA[1], R. GEOCADIN[2], N. THAKOR[1];
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Abstract: Introduction: The survival with good neurologic outcome after resuscitation from cardiac arrest (CA) is extremely low. Neurological outcome following resuscitation depends on prompt restoration of systemic circulation. In health, cerebral blood flow (CBF) is tightly controlled over a wide range of blood pressure because of cerebrovascular autoregulation (CVAR). Real time monitoring and quantification of dysfunctional CVAR after CA has not been well described. In this project, we presented CVAR via Pearson correlation coefficient between mean arterial pressure (MAP) and CBF during early resuscitation period following CA.
Methods: Eleven adult Wistar rats were randomized into two groups: low injury group (5 min asphyxia CA, n=5) and high injury group (7 min asphyxia CA, n=6). MAP (femoral arterial) and CBF (laser speckle contract imaging) combined with other vital signs including EEG and Heart rate were recorded synchronously during the whole procedure including baseline, washout, asphyxia, CPR and ROSC (return of spontaneous circulation). We studied CVAR via Pearson correlation coefficient between MAP and CBF during early resuscitation period on different levels of brain injury. A comprehensive neurological deficit score (NDS) at 4 and 24 hours were used to assess neurological outcomes in a separate cohort.
Results: There was no significant difference of pre-arrest baseline characteristics for two groups. During early ROSC phase (0-15 min post CPR), there were overshot phenomenon with both MAP and CBF in two groups. MAP peak occurred at 5.9 ± 1.3 min in low injury group; 10.5 ± 2.1 min in high injury group (p=0.02). CBF peak occurred at 5.7 ± 1.2 min in low injury group; 6.7 ± 2.3 min in high injury group (p=0.53). The EEG waveform reoccurred at 10.3 ± 2.3 min in low injury group; 16.6 ± 4.2 min in high injury group (p=0.05). CBF decreased below baseline (hypoperfusion phase) after early hyperemia, 73.9 ± 16.4 % of baseline in low injury group; 64.8 ± 13.7 % of baseline in high injury group (p=0.46). The Pearson correlation coefficient between MAP and CBF during ROSC phase was 0.48 ± 0.07 in low injury group; 0.68 ± 0.05 in high injury group (p=0.03). 4 and 24 hours post ROSC NDS score (80 best; 0 worst) were 65.8 ± 4.0

and 78.3 ± 0.8 in low injury group; 49.5 ± 2.6 and 60.0 ± 5.3 in high injury group (P<0.05). Conclusion: Cerebral perfusion after resuscitation from asphyxia cardiac arrest is characterized by early hyperemia followed by hypoperfusion. The quantitative CVAR provided an early neurological prognosis after global ischemia in adult rats. This may provide an early predictor to the injury and recovery process and present potential therapeutic window.

Disclosures: Q. Wang: None. **Y. Zeng:** None. **H. Modi:** None. **J. Senarathna:** None. **R. Geocadin:** None. **N. Thakor:** None.

Poster

215. Stroke and Ischemia I

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Program #/Poster #: 215.16/F34

Topic: C.08. Ischemia

Support: NIH Grant 5R01NS046742-17
Spanish Ministerio de Ciencia, Innovación y Universidades Grant PRX18/00241

Title: Mitophagy is activated by ischemic preconditioning

Authors: *T. JOVER-MENGUAL[1,2], H.-R. BYUN[1], J.-Y. HWANG[3], B. COURT-VAZQUEZ[1], F. PONTARELLI[1], R. ZUKIN[4];
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Abstract: Transient global ischemia arises as a consequence of cardiac arrest, cardiac surgery, profuse bleeding, near-drowning and carbon monoxide poisoning and affects ~200,000 Americans each year. Global ischemia in humans or induced experimentally in animals causes selective, delayed death of hippocampal CA1 pyramidal neurons and severe cognitive deficits. To date, there is no known treatment for the neurodegeneration associated with this devastating disease. Ischemic preconditioning is a well-known phenomenon in which a brief, sublethal ischemic insult confers robust neuroprotection to hippocampal CA1 neurons against a subsequent severe ischemic challenge. The molecular mechanisms underlying ischemic tolerance by preconditioning are not fully understood. We recently showed that global ischemia triggers a transient increase in biochemical markers of autophagy, pS317-ULK-1, pS14-Beclin-1, and LC3-II, a decrease in the cargo adaptor p62, and an increase in autophagic flux, a functional readout of autophagy, in selectively vulnerable hippocampal CA1 neurons. This is significant in that autophagy, a catabolic process downstream of mTORC1, promotes the formation of autophagosomes that capture and target cytoplasmic components to lysosomes. However, a role for mitophagy in neuronal death is as yet unknown. Mitophagy is the selective degradation of

damaged mitochondria by autophagy. Preliminary findings indicate that mitophagy is activated in reponse to global ischemia in the hippocampal CA1 pyramidal neurons *via* PINK/PARKIN/p62 signaling pathway. We further show ischemic preconditioning in normal rat (sham operated rat) induced an increase of PINK expression in the mitochondrial fraction of the CA1 neurons indicating that mitophagy can be activated by ischemic preconditioning.

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Poster

215. Stroke and Ischemia I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 215.17/F35

Topic: C.08. Ischemia

Support: NIH/NIA 1 R21 AG037843
NIH R01DC009616

Title: Visualization and assessment of stellate ganglia neronal remodeling in association with myocardial infarction (MI) and coronary artery disease

Authors: *A. G. ARAUJO[1], D. PARK[2], K. S. STEED[3], O. A. AJIJOLA[4,5], K. SHIVKUMAR[5,4], A. MAHAJAN[6,5,4], M. FISHBEIN[2], N. HAGEMAN[7], E. STARK[7], J. J. WISCO[1,7];
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Abstract: Introduction: We previously reported that there is a greater neuron density in the stellate ganglion of patients with cardiovascular disease, but the specific conditions of Coronary Artery Disease (CAD), Myocardial Infarction (MI), or Heart Failure (HF) compared with patients with little to no cardiovascular pathology has not yet been studied. We hypothesized that cardiovascular disease patients would have higher levels of neural remodeling, and perhaps more so in chronic conditions like CAD and HF as opposed to single events like MI. Methods: Stellate ganglia were harvested from one or both sides of 17 human cadavers and then serially sectioned in the coronal plane (10 µm). The ganglia were stained with luxol fast blue and cresyl violet for visualization of white matter and neurons. Prepared slides from the middle section of each ganglion were scanned digitally using Aperio Scan Scope System (Vista CA), and the neurons were counted using an automated algorithm accounting for size and shape to select for nerve cell

bodies on ImageJ. A MANOVA was performed to test the effect of heart condition type on variables of neural remodeling. Heat maps of neuron nearest neighbor density were created by convolving the centroid location for each neuron weighted by its area with a linear function, f(r), where r is the radial distance from the neuron centroid as a fraction of the maximum image dimension and f(r) = (1-5r) for r < 0.2 and 0 otherwise. Results: Analysis considering the variables of neuron size, number of neurons, and percent area of the ganglion occupied by neurons resulted in a statistical main effect of disease cohort [$F(12, 47.92)=2.085$, $p=0.0036$; Wilk's $\Lambda = 0.329$], but not side of the ganglion or the interaction of cohort and side. Between subjects effects showed that only average neuron size was a significant factor in the model [$F(4, 18098.83)=6.362$, $p=0.002$]. CAD ($p=0.005$) and MI ($p=0.014$) neurons were significantly larger, with a trend toward significance for HF neurons ($p=0.092$), than neurons in patients with little to no cardiac pathology. Heat maps correlated with these results. Conclusions: Patients with cardiovascular disease displayed neural remodeling in comparison to those with little to no cardiovascular pathology, and these effects were different across several different conditions. Some caveats to this study were sample size, especially for MI patients, and analysis of only the middle section of each ganglion. A further analysis including more of the ganglion outside of the middle section, and more subjects in each cohort, especially with MI pathology, shows promise of more definitive conclusions.

Disclosures: **A.G. Araujo:** None. **D. Park:** None. **K.S. Steed:** None. **O.A. Ajijola:** None. **K. Shivkumar:** None. **A. Mahajan:** None. **M. Fishbein:** None. **N. Hageman:** None. **E. Stark:** None. **J.J. Wisco:** None.

Poster

215. Stroke and Ischemia I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 215.18/F36

Topic: C.08. Ischemia

Support: Chinese Key R&D Plan of the State Ministry of Science and Technology 2017YFC1308403
Chinese Natural Science Foundation Grants 81870971
Chinese Natural Science Foundation Grants 81571285
Chinese Natural Science Foundation Grants 81529002

Title: Role of ASK1 in functional recovery and white matter integrity after ischemic stroke

Authors: *Y. WANG, Z. SHI, W. ZHANG, Y. GAO;
State Key Lab. of Med. Neurobiology, Inst. of Brain Sci., Fudan Univ., Shanghai, China

Abstract: Aims: ASK1(Apoptosis Signal-Regulating Kinase 1) activates both JNK and p38 MAPK pathways by phosphorylating MKK4/MKK7 and MKK3/MKK6, respectively. The inhibition of ASK1 have been shown to alleviate neuronal apoptosis following brain ischemic and neurodegenerative diseases. White matter (WM), participating in the conduction of sensorimotor information to and from cortex and determining cognitive behavior, is highly vulnerable to ischemia compared to gray matter. Besides protection of ASK1 to grey matter, the direct evidence for the role of ASK1 to WM in ischemic stroke is scant. In this study, we established transgenic mice expressing ASK1 K716R mutation (ASK1m), which inhibits the activation of ASK1, to investigate the role of ASK1 in neurological function and white matter integrity following ischemic stroke.
Methods: Transient middle cerebral artery occlusion (tMCAO) model was performed on both gender of ASK1m and wild-type C57BL/6J adult mice for 60 minutes. Sensorimotor functions were assessed by behavioral tests at pre1 and 1d, 3d, 5d, 7d, 14d, 21d, 28d, 35d after tMCAO. Morriz Water Maze was conducted from 30d to 34d for training and 35d for probe test to examine long-term cognitive function. White matter integrity was assessed by electrophysiology and immunofluorescence staining. The expression of relevant proteins was examined by western-blotting and immunofluorescence staining.
Results: Neurological functions in both gender of ASK1m mice were significantly improved and infarct volumes were reduced at 35d after tMCAO. The expression of p-ASK1 markedly decreased in ASK1m mice at 3h, 8h after tMCAO. Tunel/NeuN co-staining revealed that neuronal apoptosis in the peri-infarct region decreased in ASK1m mice at 24h after tMCAO. Compound action potentials examined on corpus callosum indicated that ASK1m preserved axonal conduction and myelin integrity at 35d after tMCAO. Also, SMI32/MBP were reduced in ASK1m mice at 35d after tMCAO. In addition, long-term cognitive function was enhanced in both gender of ASK1m mice.
Conclusions: ASK1m improves both short-term and long-term neurological functions, as well as enhancing long-term cognitive function, and significantly reduces infarct lesions following ischemic stroke. Consistently, ASK1m preserves long-term myelin integrity after ischemic stroke.

Disclosures: Y. Wang: None. **Z. Shi:** None. **W. Zhang:** None. **Y. Gao:** None.

Poster

215. Stroke and Ischemia I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 215.19/F37

Topic: C.08. Ischemia

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Title: Pre-treatment with microRNA-338 inhibitor results in increased expression of INSM1 in the hippocampus after global cerebral ischemia in rats

Authors: *B. B. GRIFFITHS, O. J. ARVOLA, A. BHUTANI, L. XU, C. STARY;
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Abstract: Global cerebral ischemia during heart attack results in decreased blood flow to the brain. The Cornu Ammonis 1 (CA1) of the hippocampus is selectively vulnerable to cell death after cerebral ischemia, while the neighboring dentate gyrus is more resilient. MicroRNAs (miRs) are short, ~23 nucleotide non-coding RNAs that downregulate mRNA through complimentary binding and subsequent degradation. Inhibiting miRs with exogenous antagomirs stereotactically injected into the hippocampus has been shown to increase the levels of target mRNAs and leads to increased protein production. In the present study, we show that the transcriptional repressor INSM1 is expressed in the DG but not the CA1 after cerebral ischemia. Treatment with miR-338 inhibitor one day before global cerebral ischemia resulted in a decrease of miR-388 expression and increase in the expression of INSM1 in the CA1. The increased INSM1 expression was present in neurons and astrocytes, but was largest in oligodendrocytes. MiR-338 inhibition resulted in increased oligodendrocyte survival through increased INSM1 expression in the rat hippocampus after global cerebral ischemia.

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Poster

215. Stroke and Ischemia I

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Program #/Poster #: 215.20/F38

Topic: C.08. Ischemia

Support: NIH VA 1I01BX003651-01A2

Title: ST2/IL-33 signaling promotes long-term recovery after cerebral ischemia

Authors: *D. XIE, H. LIU, F. XU, Q. YE, X. HU;
Univ. of Pittsburgh, Sch. of Medicine,, Pittsburgh, PA

Abstract: Objectives: Stroke is one of the most common neurological diseases. White matter injuryin the ischemic brain leads to long-term neurological deficits. ST2, a member of the interleukin 1 receptor family, and its ligand interleukin 33 (IL-33) play critical roles in immune regulation and inflammatory responses. Our previous studies have revealed that the activation of

IL-33/ST2 pathway decreased the ischemic lesion volume at acute stage after ischemic stroke. This study sought to explore the function of endogenous IL-33/ST2 signaling in white matter integrity and long-term recovery after stroke. **Method:**Transient (60 min) middle cerebral artery occlusion (tMCAO) was induced in wild type and ST2 knockout mice. Sensorimotor and cognitive functions were evaluated up to 28 days post-stroke. Immunofluorescence staining of MAP2 and MBP were used to detect brain tissue loss and white matter integrity, respectively, at 28 days after stroke. The cell death of oligodendrocyte and its precursor cells was evaluated at 3 days after stroke. **Results:**ST2 deficiency exacerbates sensorimotor deficits for at least 28 days after tMCAO, as revealed by impaired behavioral performance in adhesive removal test, rotarod test, foot-fault test and cylinder test. Cognitive functions, as evaluate by novel object recognition, Morris water-maze, and open field test, were also impaired in ST2 KO stroke mice as compared to wild type stroke mice. MAP2 staining showed increased infarct volume in ST2 KO mice vs wild type mice. Similarly, MBP staining demonstrated impaired white matter integrity at 28 days after stroke in ST2 KO mice as compared to WT mice. Consistent with deteriorated long-term white matter damage, ST2 deficiency increased oligodendrocyte and OPC cell death on 3 days after stroke. **Conclusion:**These results shed light on the IL-33/ST2 signaling as a potential immune regulatory mechanism to reduce ischemic white matter injury and restore long-term neurological functionsafter stroke.

Disclosures: D. Xie: None. **H. Liu:** None. **F. Xu:** None. **Q. Ye:** None. **X. Hu:** None.

Poster

215. Stroke and Ischemia I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

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Topic: C.08. Ischemia

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American Heart Association 17GRNT33660336
American Heart Association 19CDA34660032
Louisiana State University Research Council/Grant in Aid

Title: PYY_{3-36} affords neuroprotection through neuropeptide Y2 receptor

Authors: *C. T. CITADIN[1], C. Y. C. WU[2], H. E. POSSOIT[2], G. A. CLEMONS[1], A. COUTO E SILVA[1], R. H.-C. LEE[2], H. W. LIN[2];
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Abstract: Cardiopulmonary arrest (CA) is the leading cause of death and disability in the United States. The whole-body ischemia following CA results in subsequent brain damage causing neurological deficits. CA-induced hypoperfusion (decreased cerebral blood flow) plays a crucial

role in the progression of neuronal cell death and neurocognitive deficits after cerebral ischemia. We previously reported the enhanced activity of the sympathetic nervous system [i.e. enhancement of norepinephrine and neuropeptide Y (NPY)] contributes to CA-induced hypoperfusion. NPY is released upon activation of the sympathetic nervous system and can induce a potent vasoconstriction resulting in reduced blood supply to the brain. Our results suggest that inhibition of NPY release from presynaptic sympathetic nerves via peptide YY $(PYY)_{3-36}$, a presynaptic NPY2 receptor (NPY2R) agonist, afforded neuroprotection against CA-induced hypoperfusion, decreased neuroinflammation, decreased neuronal cell death, to result in favorable functional outcomes. Our main goal is to identify the specific NPY receptor(s) (i.e. NPY1,2,4,5R) involved in brain ischemia. A rat model of global cerebral ischemia (6 min asphyxial cardiac arrest, ACA) was used to induce CA. The presence of hippocampal mRNA of the NPY receptor subtypes (NPY 1,2,4,5 receptors) was examined by reverse transcriptase polymerase chain reaction (RT-PCR). Our results suggest that, **1**) NPY 1, 2, and 5 receptor mRNA were present in the hippocampus. To evaluate which receptors are involved in ACA and ACA + PYY_{3-36}, real-time quantitative polymerase chain reaction (RT-qPCR) was used to determine mRNA levels 1, 3, and 7 days after ACA. **2)** Since NPY2R showed significant changes under ACA + PYY_{3-36}, we attenuated the NPY2 receptor by intracerebroventricular (ICV) injection via siRNA followed by ACA. **3**) NPY2R protein expression was significant reduced (43 % lower) with ICV injection of NPY2R siRNA as compared to control animals and mRNA expression was significantly reduced with siRNA + ACA as compared to ACA alone 1 day after CA. These results suggest the importance of NPY2R in brain ischemia with a revived focus on neuropeptide Y. Future studies include assessing behavioral and memory function in the presence of PYY_{3-36} (NPY2R agonist).

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Poster

215. Stroke and Ischemia I

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Topic: C.08. Ischemia

Support: CIHR DFS-157838
NIH Grant NS056839

Title: Subventricular zone-derived neural precursor cells support tissue remodeling after ischemic cortical lesions

Authors: *M. R. WILLIAMSON[1], S. LE[1], R. L. FRANZEN[1], A. K. DUNN[1], M. R. DREW[2], T. A. JONES[3];

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Abstract: Stroke and other brain injuries cause proliferation and ectopic migration of neural precursor cells (NPCs) from the subventricular zone (SVZ) towards the site of injury. Contributions to recovery of this cytogenic response remain unclear. Using an indelible lineage tracing system, we found that photothrombotic ischemic lesions in mouse sensorimotor cortex prompted a multi-lineage migratory response; new astrocytes, neurons, and oligodendrocytes arose from SVZ-derived NPCs and localized in peri-infarct cortex. Surprisingly, at two weeks post-infarct the large majority of SVZ-derived cells in peri-cortex expressed Sox2—a marker of multipotency—suggesting that either most SVZ-derived cells remain undifferentiated or that they proliferate locally within the injury-created ectopic niche. In vivo two-photon imaging revealed that SVZ-derived NPCs in peri-infarct cortex often extend thin processes that contact nearby blood vessels. To evaluate whether NPCs influence the vascular remodeling response to injury we conditionally ablated NPCs in the adult brain of GFAP-thymidine kinase mice prior to motor cortical infarcts. Total vessel density in peri-infarct cortex of mice lacking NPCs was increased relative to mice with intact NPCs. By contrast, the density of perfused vessels in peri-infarct cortex was diminished in mice lacking NPCs. Longitudinal multi-exposure speckle imaging of cortical surface blood flow revealed that re-establishment of peri-infarct blood flow was delayed in mice lacking NPCs. Therefore, NPCs support effective vascular structural remodeling and recovery of blood flow surrounding ischemic lesions. We also monitored time- and space-resolved synaptic remodeling in superficial peri-infarct cortex with two-photon imaging of fluorescently labeled pyramidal neurons to examine whether neuronal remodeling is influenced by NPCs. Importantly, ablation of NPCs prior to motor cortical infarcts worsened recovery of skilled motor function on the single seed reaching task. Our results indicate that SVZ-derived NPCs support tissue remodeling and behavioral recovery after focal ischemic brain injury.

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Poster

215. Stroke and Ischemia I

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Program #/Poster #: 215.23/F41

Topic: C.08. Ischemia

Support: Chinese Key R&D Plan of the State Ministry of Science and Technology 2017YFC1308403
Chinese Natural Science Foundation grants 81870971, 81571285, and 81529002

Title: The neuroprotective effect and mechanism of IL13 on ischemic stroke in mice

Authors: *D. CHEN[1], P. WEI[2], W. ZHANG[3], Y. GAO[4];
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Abstract: Aims: Microglia/macrophages are activated after cerebral ischemic stroke and contribute to either brain injury or recovery by polarizing into distinctive functional phenotypes with pro- or anti-inflammatory properties. Interleukin-13 (IL-13) has been reported to polarize recovery microglia/macrophage. However, it is not clear whether IL-13 is benefiting for the long-term outcomes after stroke and the underlying mechanisms. In the present study, we investigated the effect of IL-13 on long-term recovery and microglia/macrophage polarization in a transient middle cerebral artery occlusion (tMCAO) model.
Methods: tMCAO was induced in adult male C57BL/6J mice. IL-13 (60ug/kg) was administered intranasally 2 hours and 1 - 7 days after tMCAO. Sensorimotor functions (rotarod, corner, adhesive remove and foot fault) as well as spatial learning and memory function (Morris water maze) were assessed until 35 days after tMCAO. Brain infarct volume was analyzed at 35 days after tMCAO. And the white matter integrity was evaluated by electrophysiology and immunofluorescence staining. Microglia/macrophage polarization were assessed using immunofluorescence staining.
Results: The results showed that IL-13 treatment significantly improved overall sensorimotor outcomes than the vehicle mice after tMCAO, as revealed by increased latency to fall off rotarod, improved asymmetry of corner test, decreased latency of adhesive removal and decreased number of foot faults. IL-13 treatment enhanced long-term cognitive functional recovery in the Morris water maze. IL-13 treatment decreased neuronal tissue loss at 35 days. IL-13 treatment contributed to the improved white matter integrity with improved compound action potential (CAP) and increased myelin basic protein (MBP). IL-13 treatment decline the expression of CD16 and inhibit the activation of proinflammatory microglia/macrophages.
Conclusions: IL-13 may play a neuroprotective role by altering the polarization of microglia/macrophages, reducing white matter damage and improving neurological deficits after ischemic stroke. These results suggest that immunomodulation with IL-13 is a promising approach to promote long-term functional recovery after stroke.
Key Words: interleukin-13, white matter, microglia/macrophage, neurological function, ischemic stroke

Disclosures: D. Chen: None. **P. Wei:** None. **W. Zhang:** None. **Y. Gao:** None.

Poster

215. Stroke and Ischemia I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 215.24/F42

Topic: C.08. Ischemia

Support: DGAPA-PAPIIT IN226617
CONACYT A1-S-13219

Title: Proteomic characterization and protective effect against brain ischemia produced by adult mouse neural progenitor cells-derived extracellular vesicles

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Abstract: Hypoxia produced by stroke activates the proliferation of neural progenitor cells (NPC) in the subventricular zone of the adult mouse brain. Newly generated cells seem to participate in the protection of the ischemic brain through the release of extracellular vesicles (EVs) that might target different cell types and modify their function by delivering proteins, lipids, and nucleic acids. In this study, we investigated the possible communication route mediated by EVs between NPC and neurons under conditions of cerebral ischemia using an *in vitro* model of oxygen and glucose deprivation (OGD). We collected OGD-activated NPC-derived EVs by differential centrifugation of the conditioned media in which NPC were grown for 12 h after OGD, and as a control, we harvested EVs released by cells cultured under normoxic conditions. EVs were characterized by shape and size with transmission electron microscopy and NanoTracking assays, and the presence of typical exosome markers was assessed by western blot. The proteomic profiling of NPC-derived EVs was carried out after resolving the proteins by polyacrylamide gel electrophoresis and performed in-gel digestion with trypsin, then the identity of the products was determined by tandem mass spectrometry (ESI-IMS-MS). Overrepresentation analyses of the proteomic data allowed us to identify executioners of signaling pathways involved in neuroprotection. Among the identified regulators we found β-catenin, the effector of the Wnt pathway which has been previously shown to mediate neuronal survival through the activation of protein kinase B (PKB/Akt). To test the neuroprotective effect of NPC-derived EVs against noxious stimuli relevant in brain ischemia, such as OGD, excitotoxicity, apoptosis and oxidative stress, rat primary cortical neurons were exposed to EVs for 22 h after which neuronal viability was determined. Overall, we found that NPC-derived EVs were able to reduce the neurotoxicity elicited by OGD and excitotoxicity. In addition, adult male mice were subjected to occlusion of the middle cerebral artery for 50 min; animals were then

injected i.c.v. with a suspension of NPC-derived EVs and brain tissues were evaluated after 24 h. NPC-derived EV reduced the stroke lesion and improved motor function. These results indicate that NPC-derived EV have a protective effect on the ischemic cascade activated by OGD and stroke, and that this could be mediated by the molecular mediators of intracellular signaling pathways that are carried within them. These findings hold potential therapeutic value to treat ischemia-related diseases.

Disclosures: A.N. Campero-Romero: None. **E. Ríos-Castro:** None. **L. Tovar-y-Romo:** None.

Poster

215. Stroke and Ischemia I

Location: Hall A

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Topic: C.08. Ischemia

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Title: Uptake, distribution and fate of extracellular vesicles in ischemic retina

Authors: *L. M. ELLYTHY[1], B. MATHEW[1], L. TORRES[1], S. TRAN[1], N. MUCKOM[1], M. CHENNAKESAVALU[1], L. GAMBOA ACHA[1], S. RAVINDRAN[2], S. MEHREEN[2], S. ROTH[1]; [1]Anesthesiol., [2]Oral Biol., Univ. of Illinois at Chicago, Chicago, IL

Abstract: Retinal ganglion cell (RGC) loss is a major cause of vision impairment/loss and a common underlying mechanism associated with glaucoma, diabetic retinopathy, central retinal artery occlusion and optic neuropathy in association with multiple sclerosis (MS). We have previously reported the robust neuroprotective effect of mesenchymal stem cell-derived extracellular vesicles (EVs) in retinal ischemia. Retinal neurons endocytosed EVs in a dose-dependent, saturable manner mediated by Heparin sulfate proteoglycan (HSPG) receptors via caveolin. When EVs are endocytosed by target cells, their protein, microRNA (miRNA, or miR), and mRNA cargo trigger specific responses, including regenerative properties. However, it is not clear whether the therapeutic effect of EVs in the retina is directly on the RGCs or if there is an orchestrated interplay of other cell types in this effect. Accordingly, it is essential to determine the time course of EVs reaching the retina and the fate of injected EVs in the vitreous. Therefore, the purpose of this study was to evaluate the fate of fluorescently labeled

intravitreally injected EVs in both normal and ischemic eyes of rats. EVs ($1x10^9$/4 µl), or PBS (4 µl) were injected into the vitreous humor of both the ischemic (right)and non-ischemic (left) eyes, 24 h after retinal ischemia. Fluorescent fundus images were obtained using a Micron IV Retinal Imaging at 1, 3, 7, 14, and 28 days after injections into the vitreous humor. We analyzed EV distribution within the vitreous and later to the retina throughout a one-month period post administration. Furthermore, immunofluorescent imaging of retinal cryosections was performed using antibodies for different cell types to determine cell specificity and time course. Based on observation, EVs in the vitreous moved into the retina gradually and were visible in the retina even at 4wks. Confocal imaging of retinal sections demonstrated uptake of EVs by retinal neurons, retinal ganglion cells (RGCs)and microglia. In addition, exploration of the binding and unbinding kinetics of exosomes to proteins with the vitreous was performed which demonstrated no significant difference between the normal and ischemic eye in terms of EV binding. Together the findings of this study will allow us to understand the importance of enhancing the specificity of the EVs to target cells for prolonging their action and to determine whether multiple injections would be required for successful therapy.

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Poster

215. Stroke and Ischemia I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

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Topic: C.08. Ischemia

Support: German Research Foundation (DFG, LI2534/2-1)
European Research Council (ERC-StG 802305)
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Title: The role of short-chain fatty acids in post-stroke regeneration

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Abstract: The gut harbours a readily adapting bacterial population known as the gut microbiome (GM). The GM has been shown to alter the outcome of many brain diseases including stroke.

Previously we and others have observed a bi-directional interaction of the GM and brain. The immune system was identified as the key mediator along the gut-brain axis which affected stroke outcome. Stroke-induced GM dysbiosis is evident for many weeks, however, the implications and mechanistic alterations for long-term recovery after stroke are currently unknown. Within this study we focus on short-chain fatty acids (SCFA) as one of the key bioactive metabolites derived from the GM. We administered SCFA supplementation in drinking water, and investigated role of these metabolites within the recovery phase after stroke. Experimental stroke was induced by photothrombotic lesions to the motor cortex of adult mice. Reorganization of cortical networks and long-term functional recovery after stroke is a multicellular process encompassing neuronal plasticity, resident immune cells and brain-invading cells. Using *in vivo* calcium imaging of Thy1GCaMP6s mice, we observed changes in dynamics of contralateral cortex connectivity, following SCFA supplementation. This was associated with improved motor function as assessed by an automated lever-pull test. Furthermore, SCFA treatment significantly altered dendritic spine densities as well as synaptic counts of excitatory neurons in the cortex. RNA sequencing of the cortex indicated the potential involvement of microglia cells and synaptic maintenance as genes associated with phagocytosis were highly upregulated. Additionally, using an automated morphology analysis of cortical microglia we found more ramified structures, hinting at reduced microglia activation compared to controls. By flow cytometry, we characterised the immune cell populations within the brain as a potential source of neuroinflammation. SCFA supplementation reduced the cerebral lymphocytes after stroke. This was also evident in the spleens, suggesting SCFA were acting initially in the peripheral compartment. Using RagKO mice, we found that in the absence of T and B lymphocytes, microglia did not change morphology under SCFA supplementation. Taken together these results suggest that microbiota-derived SCFA are important for improved post-stroke recovery, and that modulation of peripheral immune cells by SCFA before brain infiltration potentially affects microglia-driven synaptic reorganization after stroke. Finally, this presents the GM and metabolites in a new light, as a probable performer and therapeutic target within the recovery phase after stroke.

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Poster

215. Stroke and Ischemia I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 215.27/F45

Topic: C.09.Stroke

Support: NIH

Title: W2476, a novel thiordoxin interacting protein inhibitor attenuates post stroke hemorrhagic conversion in hyperglycemia mice subjected to thrombolytic therapy

Authors: L. LI, S. ISMAEL, ***A. YOO**, T. ISHRAT;
Dept. of Anat. and Neurobio., The Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Stroke patients with acute hyperglycemia (HG) manifest higher mortality and poorer outcomes after tissue plasminogen activator (tPA) therapy, partially due to deteriorated blood-brain barrier (BBB) disruptions and intracranial hemorrhages (ICH). Thioredoxin-interacting protein (TXNIP) is an endogenous inhibitor of the thioredoxin (TRX) pathway, known to be induced in HG mice upon cerebral ischemia and reperfusion. We hypothesize that a novel TXNIP inhibitor, W2476 should ameliorate post-stroke brain endothelial injuries associated with acute HG and tPA treatment. Acute HG *in vivo* was generated and maintained by intraperitoneal injections with dextrose solution. After thromboembolic middle cerebral artery occlusion (MCAO) mice were treated with W2476 suspension (400mg/kg) via an intragastical gavage, followed by an intravenous tPA infusion (10mg/kg) at post-stroke hour 3. Mice were sacrificed 24 hours after MCAO. In-vitro, bEnd3 cells were grown in high glucose medium (33mM glucose) and subjected to oxygen-glucose deprivation (OGD) for 8 hours (0.1% O2, 94.9% N2, 5% CO2), followed by an exposure to medium containing tPA (20μg/ml) and high glucose for 16 hours. Cells were pretreated with 15μM W2476 before OGD. In contrast to normoglycemic mice, significant increases were determined in HG mice in terms of infarct size, ipsilateral hemoglobin (Hb) excess, ipsilateral expression of ZO-1, Occludin, TXNIP, TRX, interleukin-1β (IL-1 β), and vascular endothelial growth factor B (VEGFB) proteins. The tPA treatment upon acute HG demolished all those increases except the Hb excess, which was not significantly corrected until W2476 was applied. The viability of bEnd3 cells was likewise compromised upon OGD followed by tPA and HG exposure, whereas treatment with W2476 revived these brain-derived endothelial cells. Acute HG exacerbates neurovascular injuries upon ischemic stroke, whilst tPA alone fails to diminish damages in brain vessels. TXNIP inhibition reduces endothelial vulnerability and has therapeutic potentials against post-ischemia hemorrhagic transformation associated with acute HG and tPA-induced reperfusion. Underlying pharmacological details need to be further elucidated.

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Poster

215. Stroke and Ischemia I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 215.28/F46

Topic: C.09.Stroke

Support: AG042189
NS074895

Title: Gut microbiome modulates the effects of estrogen treatment in female rats

Authors: *S. PANDEY[1], M. PARK[1], R. PILLA[1], A. PANTA[1], F. SOHRABJI[2];
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Abstract: Our previous studies have shown that estrogen treatment to adult females is neuroprotective for stroke while estrogen treatment to reproductive senescent (RS, middle-aged, acyclic) females exacerbates stroke injury. The mechanism underlying the paradoxical effects of estrogen on stroke recovery on the two age groups are poorly understood. Recent studies have shown a reciprocal interaction between stroke and the gut microbiome. Specifically, stroke can cause gut dysbiosis, and transfer of 'young' microbiome can improve stroke outcomes in aged animals. To determine whether estrogens neuroprotective effect is mediated via the gut microbiome, we tested the effect of fecal microbiome transfer (FMT) from estrogen-treated adult female rats on stroke outcomes in estrogen-treated middle-aged female rats. Two sets of animals were prepared: *Donor*: Adult (5-6 month) and RS (10-12 month) female rats were ovariectomized (OVX) and replaced with estradiol (1mg/pellet; 60-day release). *Recipient*: A separate set of OVX and estradiol replaced RS females were prepared. Three weeks later, the recipient rats received 2 consecutive days of antibiotic by oral gavage (0.5 mls of a 0.3g/ml streptomycin HCL solution), to eliminate existing gut bacteria, followed by oral gavage of fecal microbiome transfer (0.5ml of donor sample/day) from either estrogen-treated adult rats (Ad OVX+E) or estrogen-treated RS rats (RS OVX+E) for 5 days. Recipient rats were subjected to MCAo 24 hours after their final gavage. Fecal samples collected before and after (2d and 5d) stroke were analyzed by Illumina-sequencing of the 16S rRNA gene. Animals were terminated at 5d and the brain was processed for infarct volume. Our results show that infarct volume was significantly decreased in estrogen-treated middle-aged rats that received FMT from Ad OVX+E females compared to FMT from RS OVX+E females and had lower levels of interleukin 17 at 2d after stroke. However, there were no group differences in gut dysbiosis as determined by the ratio of Firmicutes to Bacteroidetes (F:B), although there was an elevated abundance of S24-7 in the group that received RS OVX+E FMT. Collectively, these data indicate that microbiome transfer from the healthy adult rats to middle-aged rats improves stroke outcomes without improving gut dysbiosis, suggesting that FMT from Ad OVX+E results in transfer of either specific beneficial bacteria or transfer of beneficial gut metabolites (such as SCFAs).

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Poster

215. Stroke and Ischemia I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 215.29/G1

Topic: C.09.Stroke

Support: AG042189
NS074895

Title: Gut dysbiosis after ischemic stroke may contribute to sex differences in stroke outcomes in adult rats

Authors: *Y. EL-HAKIM, K. MANI, A. ELDOUH, F. SOHRABJI;
Neurosci. and Exp Therapeut., Texas A&M Hlth. Sci. Ctr., Bryan, TX

Abstract: Sex differences in stroke have been well-documented with young adult males having a higher risk for and a worse outcome after a stroke event than young adult females. This has been attributed to the neuroprotective and immunomodulatory properties of the gonadal steroid estrogen. Estrogen has been shown to act directly on neurons to promote their survival, as well as the blood brain barrier and immune cells. Recent evidence indicates that the gut microbiome may play an important role in stroke outcomes. Increased gut permeability in response to stroke is immediate and significant, as well as changes in gut microbiota. Here we tested the hypothesis that males have a greater permeability of the gut blood barrier and experience gut dysbiosis in response to stroke. Male and female Sprague Dawley rats (5-7 months of age) were subject to endothelin-1 induced middle cerebral artery occlusion (MCAo) simulating an ischemic stroke event. Sensory motor tests were conducted pre, 2d and 5d after MCAo. Blood and fecal samples were also obtained at the same time points and tested for the mucoprotein, mucin-1. We observed that both males and females show an early and transient increase in mucin-1 levels in serum at 2d post MCAo, which return to basal (pre-stroke) levels at 5d post MCAo. This is indicative of an increase in gut blood permeability in the acute phase of stroke, but no sex difference was observed. In contrast, fecal levels of mucin-1 were significantly lower in males as compared to females, especially after stroke. The low levels of mucin-1 in male fecal samples is likely due to the loss of mucolytic bacteria, the most common being akkermansia muciniphila, a microbe implicated in improved outcome in cardiovascular disease. Akkermansia muciniphila produces high levels of SCFAs, which is also shown to improve stroke outcome. These data indicate that gut dysbiosis may be an underlying driver of sex differences in stroke outcome.

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Poster

215. Stroke and Ischemia I

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Topic: C.09.Stroke

Support: CIHR, CCNA and CFI operating and infrastructure grants
Kathleen & Dr Henry Barnett Research Chair in Stroke Research
Western University Medical and Health Sciences Research Board Seed Research Grant
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Opportunities Fund of the Academic Health Sciences Center Alternative Funding Plan of the Academic Medical Organizationof Southwestern Ontario (AMOSO)

Title: Temporal progression of cardiac inflammation post-insular stroke in the rat

Authors: *V. JAREMEK[1], S. IANKOV[1], T. MILAZZO[1], S. MYERS[1], L. WANG[1], S. N. WHITEHEAD[1], L. A. SPOSATO[2];
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Abstract: Patients who have suffered an ischemic stroke with damage to the insular cortex (IC) frequently develop post-stroke myocardial complications. However, the pathophysiology of these post-stroke cardiac changes requires additional investigation which is complicated by a lack of pre-clinical models. Further, whether post-stroke cardiac dysfunction occurs in parallel with autonomic dysregulation and systemic inflammation following stroke is unknown. IC ischemic stroke was induced in 6-month-old male Wistar rats via unilateral stereotaxic injection of endothelin-1 (ET-1) into right or left IC (n=6/surgical group). Control rats received a phosphate-buffered saline (PBS) injection. To establish a time course of cardiac dysfunction, hearts were histologically examined at 6, 24 hours, 7, 14 and 28 days post-stroke for fibrosis (Masson's Trichrome stain) and inflammation (CD68, CD3, CD45, myeloperoxidase, CD45R, immunostaining). Areas of interest included the 4 heart chambers and left atrial/pulmonary vein border (LA-PV border), an area of enriched autonomic innervation. Blood samples were also collected at respective timepoints for BNP and TNT ELISA analysis as well as cytokine multiplex analysis. Brains were histologically assessed for infarct volume via thionin staining for Nissl substance. In addition, brainstem areas relay autonomic information to the heart, thus OX-6 immunostaining was performed to identify activated pro-inflammatory microglia. Brainstems were analyzed at the level of the medulla, pons and midbrain. Results from this study showed that focal IC ischemic stroke led to neutrophil infiltration at 6 hours. Left atrial tissue

experienced long-term fibrosis at 28 days following focal IC stroke. Further, B lymphocyte infiltration was seen at 28 days post-stroke in left ventricular tissue. Preliminary results show increased infarct volume in ET-1 rats as well as suggest increased brainstem neuroinflammation, specifically at the nucleus of the solitary tract 28 days post-stroke. These findings provide insight into the progression of post-stroke cardiac changes and suggest that inflammation is a treatable target to prevent post-stroke myocardial injury.

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Poster

216. Stroke and Ischemia II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 216.01/G3

Topic: C.09.Stroke

Support: TOYOTA Motor Corporation grant

Title: Electroencephalographic synchrony measure reflects recovery of post-stroke aphasia

Authors: *T. KAWANO[1,2,4], N. HATTORI[2,3,1,4], Y. UNO[4], M. HATAKENAKA[1], H. YAGURA[1], H. FUJIMOTO[1], T. YOSHIOKA[1], M. NAGASAKO[1], H. OTOMUNE[1], H. MOCHIZUKI[2], K. KITAJO[4,5,6], I. MIYAI[1];
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Abstract: Objective: Biomarkers for post-stroke impairment and recovery are attracting attention to researchers and clinicians. Resting-state functional connectivity (FC) is a promising biomarker and Siegel et al. reported that FCs between homotopic interhemispheric regions were associated with various clinical scores. We reported similar association by using an EEG synchrony measure: the phase synchrony index (PSI). In our previous cross-sectional study, the PSI between bilateral inferior frontal lobes (motor language area and its right homotopic region) was correlated with the clinical score for post-stroke aphasia. The aim of this exploratory longitudinal study was to evaluate the potential of the PSI as a biomarker for aphasia recovery. **Methods:** Participants were patients presenting with aphasia due to ischemic stroke in the left

hemisphere undergoing inpatient rehabilitation. Language functions were assessed by the Standard Language test of Aphasia (SLTA). Aphasia Rating Scale speech score (ARSsp; range: 0-70) was calculated based on the SLTA subscore. The PSI (β band) between corresponding inferior frontal lobe was computed from resting state EEG (19-channel) data (Kawano et al. NNR, 2017). Stroke lesions were manually drawn on MRI, and were spatially normalized to the Montreal Neurological Institute stereotaxic space to compute stroke lesion volume (LV). EEG measurement and clinical assessment were performed at admission and discharge (PSI1/2 and ARSsp1/2, respectively). Then, we evaluated correlation between the changes in the PSI and aphasia recovery assessed by the ARSsp recovery ratio [(ARSsp2-ARSsp1) / (70 - ARSsp1)].
Results: Seventeen patients (mean age: 70.4, five females, mean time post-stroke: 33.6 days, median stroke LV: 57,744 mm^3) were enrolled. Median interval period of SLTA assessment was 57 days. Median ARSsp1 and ARSsp2 were 12.5 and 41.5, respectively. ARSsp2 was significantly higher than ARSsp1 (P = 0.001). The PSI2 was significantly higher than PSI1 (P = 0.022). LV was not correlated with ARSsp recovery ratio (P = 0.15). In contrast, PSI1/PSI2 ratio was significantly positively correlated with ARSsp recovery ratio (P = 0.017). Partial correlation analysis (non-parametric) revealed that this correlation was significant (P = 0.018) after adjusting for the effect of stroke LV.
Conclusion: The PSI between bilateral motor language area increased alongside with rehabilitation and the PSI ratio was correlated with improvement of speech score, independent of stroke LV. Homotopic EEG synchrony between inferior frontal lobes may be a biomarker for aphasia recovery following stroke.

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Poster

216. Stroke and Ischemia II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 216.02/G4

Topic: C.09.Stroke

Support: JSPS KAKENHI 17K10422

Title: Difference between twice and single refocus spin-echo diffusion sequence in early phase of stroke patients

Authors: *K. NAKAMURA, S. MINAKATA, H. TOYOSHIMA, T. KINOSHITA;
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Abstract: [Introduction] Susceptibility-induced background magnetic field interact to diffusion weighted MRI signal intensity, especially with typical single refocused pulsed-gradient spin-echo (SRSE-DWI) sequence. On the other hand, twice refocused spin-echo (TRSE-DWI) sequence reduces the effects. The difference image of the two DWI sequences should be an image reflecting tissue susceptibility. Tissue susceptibility reflects property in brain ischemic lesions such as oxygen consumption, vessel dilatation, deoxy-hemoglobin concentration and so on. Therefore, apparent diffusion coefficient (ADC) difference between SRSE-DWI and TRSE-DWI might be useful for diagnosis of the stroke patient. The purpose of this research is to examine the property of the difference image of two DWIs in early phase of stroke patients.[Methods] Nine patients with cerebral infarction in the penetrating branch area were included. MRI was examined within two weeks after stroke onset with a 3T MRI. SRSE-DWI and TRSE-DWI were acquired with TR / TE = 6000/72 ms and 5 mm slice thickness. ADC were calculated from DWI of b values 0 and 1000. Difference image (ADC-Diff) between ADC of TRSE (TRSE-ADC) and SRSE (SRSE-ADC) was evaluated. For the evaluation of the healthy contralateral hemisphere, brain segmentation was processed by the Freesurfer v6.04 using anatomical T1-weighted image acquired by 3D MPRAGE sequence. Coregistration to DWI from structural image was also processed by the Freesurfer software. For the evaluation of ischemic brain region, region of interest (ROI) was manually selected surrounding the ischemic region. [Results and Discussion] Even almost same contrast in SRSE-DWI and TRSE-DWI sequence, ADC-Diff was large in the place where the larger magnetic susceptibility effect exists such as the pallidum and striatum. It is reasonable that the difference image reflects the change in magnetic susceptibility. ADC-Diff in ischemic brain region show higher value in the periphery of cerebral infarction. The biggest difference in ADC-Diff was shown at ADC around $0.6\times10^{-3}mm^2/s$. Identification of ischemic core was an ADC under $0.62\times10^{-3}mm^2/s$ in previous literature. ADC-Diff results may reflect the change in magnetic susceptibility associated with inflammation and vasodilation. DWI is indispensable for clinical diagnosis and obtain the two DWI images in a short time. Tissue magnetic susceptibility reflected image of ADC-Diff could be clinically useful for the stroke patient diagnosis.

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Poster

216. Stroke and Ischemia II

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Topic: C.09.Stroke

Support: NIH Grant 5KL2TR001999
Roberta K. Courtman Revocable Trust

Title: Characterizing changes in neural activity and movement kinematics during an object-manipulation task in patients who regained motor function after stroke

Authors: *K. A. MAZUREK[1], S. MIZOBUCHI[2], E. PATELAKI[2], D. RICHARDSON[2], N. ABRAHAM[2], S. HOFFMAN[2], A. C. BUSZA[3], J. J. FOXE[1], E. G. FREEDMAN[1];
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Abstract: When reaching to grasp specific objects, neural activity in posterior parietal cortex communicates information about the object's location and shape to premotor cortex for developing plans for reaching and grasping. These motor plans are then communicated to primary motor cortex which coordinates execution of the movements. After a stroke, some patients lose the ability to perform such skilled movements and become dependent on caregivers to perform daily tasks. Other patients have initial weakness but then regain motor function. How regained motor function relates to neural reorganization post-stroke is not well understood. Some patients might regain motor function because some portion of the impaired brain region recovers function. Other patients might utilize unaffected neural circuits to compensate for the impaired brain region. Current rehabilitation therapies do not take into account where the stroke occurred or which neural pathways are affected when rehabilitating motor function.
For healthy participants and patients who regained motor function after stroke, we compared neural activity and movement kinematics during an object-manipulation task. Utilizing Mobile Brain/Body Imaging (MOBI) techniques, we recorded neural activity using high-density electroencephalography (EEG) and reaching kinematics using motion capture technologies for comparison between the two cohorts. Different sensory information was used as instructions for manipulating specific objects which allowed for identifying how neural activity changes in motor cortex and posterior parietal cortex when performing the same actions. The use of MOBI technologies provided a framework for comparing the neural control of movements in patients with a stroke in which sensorimotor integration is impaired due to interruption of healthy communication between brain regions. Such a framework is a first step towards understanding recovery at a neural systems level that ultimately might lead to rehabilitative systems that restore function more efficiently.

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Poster

216. Stroke and Ischemia II

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Wellcome Trust (094874/Z/10/Z)
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Title: No evidence for cortical reorganization after subcortical stroke using resting state fMRI

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Abstract: After a stroke, patients can often show substantial recovery from upper limb paresis. Cortical reorganization, defined as behaviorally relevant changes in structural or functional connectivity between areas adjacent and remote from the infarct, has been considered a mechanism for stroke recovery. An increasingly popular approach for attempting to detect such changes in connectivity is to determine the correlation of BOLD (blood oxygenation level dependent) activities between brain areas using resting state functional magnetic resonance imaging (fMRI). Using this method, studies have reported increased correlations either within the motor areas of the lesioned hemisphere or between the lesioned and non-lesioned hemispheres in the setting of unilateral stroke. Here, we report a longitudinal resting state study in 19 patients with subcortical stroke. Patients and controls were scheduled for five scanning sessions over 12 months, starting one week after stroke. We found no systematic intra- or inter-hemispheric differences in connectivity between cortical motor areas when comparing patients with controls at any time point. A corollary of this was that patients' connectivity did not change over time despite substantial improvements in upper limb function. Thus, there was no evidence for recovery-related post-stroke cortical reorganization using resting state methods. These results could be considered a caution with regard to resting state but here we argue instead that they are consistent with other reasons to question the idea of recovery-related cortical reorganization after stroke.

Disclosures: M. Branscheidt: None. **N. Ejaz:** None. **J. Xu:** None. **M. Widmer:** None. **M.D. Harran:** None. **J. Cortes:** None. **T. Kitago:** None. **P.A. Celnik:** None. **S. Mori:** None. **A.V. Faria:** None. **C. Hernandez-Castillo:** None. **J. Diedrichsen:** None. **A.R. Luft:** None. **J.W. Krakauer:** None.

Poster

216. Stroke and Ischemia II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 216.05/G7

Topic: C.09.Stroke

Support: 1K01HD091283

Title: Ipsilesional hippocampal volume is directly associated with motor performance in chronic stroke patients: An ENIGMA stroke recovery analysis

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Abstract: The hippocampus is fundamental to cognition and may play a role in motor skill learning. In the context of stroke motor rehabilitation research, however, this brain region has not been widely studied. Hippocampal volumes are known to decrease with age and cognitive impairment. The goals of this study were to: 1) examine age-related effects on hippocampal volumes in the ENIGMA Stroke Recovery multi-site database, and 2) assess the relationship between hippocampal volume and post-stroke motor performance. We hypothesized that

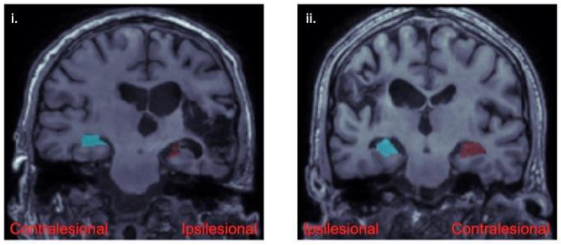
hippocampal volume is inversely associated with age and directly associated with motor performance in chronic stroke patients. We processed T1-weighted volumetric brain MRI scans (N=253 chronic stroke patients across 10 research sites; Figure 1A) with FreeSurfer 6.0 to segment hippocampal volume. Failed segmentations were excluded (Figure 1A-B). We used mixed effects linear models to test ipsilesional and contralesional associations between hippocampal volume and age. Research site was included as a random effect, along with fixed effects of sex, intracranial volume (ICV), and lesioned hemisphere. To examine the impact of motor performance on hippocampal volumes, each model was tested with and without including motor performance as a fixed effect. As expected, hippocampal volume was inversely associated with age for both the ipsilesional and contralesional hippocampi (Figure 1C-i,ii). Motor performance was directly associated with ipsilesional hippocampal volume (Figure 1C-iii) but not contralesional (Figure 1C-iv). Similarly, including motor performance in the model significantly improved the model fit only in the ipsilesional (ΔAIC= -16.7) but not the contralesional model.

These findings demonstrate that hippocampal volumes decline with age after stroke and larger ipsilesional post-stroke hippocampal volume is linked with better motor performance. Future studies examining cognitive and motor scores together are needed to parse out the functional impact of hippocampal volume in stroke.

(A)

	N	% QC Fail	Age (years)	M/F	Avg Motor Performance
Ipsilesional	164	35.2%	60.7±11.8	113/51	0.73±0.30
Contralesional	183	27.7%	59.1±12.3	127/56	0.71±0.31

(B)



(C)

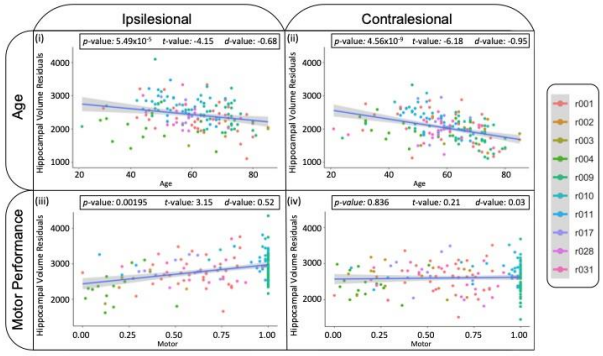


Figure 1: (A) Ipsilesional and contralesional hippocampal volumes were analyzed separately and had different sample sizes due to the exclusion of failed segmentations. Motor performance was measured using different motor assessments at each site; we normalized all motor scores as a percentage of the maximum possible score (range: 0 to 1), where 1 indicates no deficit.(B) FreeSurfer 6.0 was used to segment the hippocampus. Each hippocampus was visually inspected for quality and excluded if necessary. B-i shows an example of an ipsilesional hippocampus (red) that was not successfully segmented and was subsequently excluded from the analysis. The contralesional hippocampus (blue), however, was well segmented and included in the contralesional analysis. B-ii shows an ipsilesional hippocampus that was successfully segmented. (C) Both the ipsilesional (C-i) and contralesional (C-ii) hippocampal volumes were inversely associated with age. Motor performance was directly associated with hippocampal volume in the ipsilesional hemisphere (C-iii), but not the contralesional hemisphere (C-iv). The legend indicates research site.

Disclosures: **A. Zavaliangos-Petropulu:** None. **B. Bigjahan:** None. **M.R. Borich:** None. **T.R. Brown:** None. **C.M. Buetefisch:** None. **W.D. Byblow:** None. **S.C. Cramer:** None. **A. Dula:** None. **K. Gill:** None. **A. Goud:** None. **D.H. Hwang:** None. **N. Khoshab:** None. **H. Kim:** None. **A. Kuceyeski:** None. **C.E. Lang:** None. **M. Lotze:** None. **B.J. MacIntosh:** None. **A. Ramos-Murguialday:** None. **A.D. Robertson:** None. **P. Roberts:** None. **M.S. Shiroishi:** None. **C.M. Stinear:** None. **R.C. Craddock:** None. **K.A. Wong:** None. **G. Thielman:** None. **N.S. Ward:** None. **G.F. Wittenberg:** F. Consulting Fees (e.g., advisory boards); On advisory board of Battelle Memorial Foundation. **N. Jahanshad:** None. **P.M. Thompson:** 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.; PT is partially supported by Biogen for work unrelated to this research. **S. Liew:** None.

Poster

216. Stroke and Ischemia II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 216.06/G8

Topic: C.09.Stroke

Support: Sir Henry Dale Fellowship (Wellcome Trust/Royal Society) (102584/Z/13/Z)
Wellcome Trust (203139/Z/16/Z)
NIHR Biomedical Research Centre, Oxford.

Title: Using a novel EMG controlled force tracker task to study relationships between motor learning and multimodal MRI brain characteristics in chronic stroke

Authors: *E. L. HINSON[1], M. K. FLEMING[1], F. VAN HORSSEN[1], A. POGOSYAN[2], W. T. CLARKE[1], C. J. STAGG[1];
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Abstract: Background
Motor rehabilitation for stroke survivors is frequently informed by motor learning in healthy controls, however it is not fully established if stroke survivors learn motor skills in the same way, potentially questioning the validity of basing rehabilitation on these models. Furthermore, tasks can be limited by floor/ceiling effects. Here, a novel adaptation of a Force Tracker Task is tested in chronic stroke survivors and age-matched controls. Relationships between task performance and brain structure, function and neurochemical systems are studied using multimodal 3T MRI.
Methods
Chronic stroke survivors (> 6 months post stroke) and age matched controls completed a 3T MRI scan, followed by an EMG controlled Force Tracker Task (EMG-FTT). The MRI consisted of

structural, functional and spectroscopic imaging.
In the EMG-FTT task, modulated contraction and relaxation of the forearm extensor muscle (affected/non-dominant arm) was used to control cursor position and track a moving target. Participants completed an evaluative questionnaire (5-point Likert scale) and Visual Analogue Scales for sleepiness and muscle fatigue.
Upper limb function was assessed in all participants using the Action Research Arm Test (ARAT) and the 9-Hole Peg Test (9-HPT).

Results

Preliminary data (n= 4 patients, 4 controls) suggest that participants understood the task goal, with no between group differences in evaluation of task difficultly. There tended to be no between group differences in ratings of ability to concentrate on the task, or evaluation of task induced fatigue.
Tracking error (distance from target to cursor) was continuously recorded and both groups were able to perform the task. Change in performance was calculated as change in mean error during training. Residual functional impairment of the affected arm did not prevent task performance for stroke survivors, however ARAT score on the task-performing arm (affected/non-dominant arm) tends to be associated with performance (mean absolute error) on the first task block.

Discussion

Motor learning tasks in stroke and age matched cohorts are often limited by floor/ceiling effects. The EMG-FTT is not reliant on a minimum residual functional ability and can therefore assess learning ability in a wide range of participants. Although initial indications suggest a relationship exists between initial task performance and ARAT score, this does not influence improvement over the course of task performance. Understanding the process of motor learning further, and specifically if/how this changes post-stroke is crucial to the development of the most effective rehabilitation strategies.

Disclosures: E.L. Hinson: None. **M.K. Fleming:** None. **F. Van Horssen:** None. **A. Pogosyan:** None. **W.T. Clarke:** None. **C.J. Stagg:** None.

Poster

216. Stroke and Ischemia II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 216.07/G9

Topic: C.09.Stroke

Title: Time-varying functional connectivity in acute ischemic stroke

Authors: *A. K. BONKHOFF[1], F. A. ESPINOZA[2], H. GAZULA[2], L. HENSEL[1], A. REHME[1], L. VOLZ[1], G. R. FINK[1,3], V. CALHOUN[2,4,5], C. GREFKES[1,3];
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Abstract: Background: Ischemic stroke leads to sudden focal brain damage and thereby severely disrupts structural and functional anatomy. Various imaging techniques have already furthered our understanding of these processes, particularly by highlighting potential contributions of individual brain areas to the recovery of function. This work expands current literature by presenting novel findings on the unique time-varying characteristics of functional connectivity in stroke patients.
Methods: Our analysis relied on resting-state fMRI data of 12 acute ischemic stroke patients and 13 age- & gender-matched healthy controls (3T, TR: 0.7 s, 567 volumes). Due to our focus on motor symptoms, we concentrated on the sensorimotor, subcortical and cerebellar domains as available from Allen and colleagues (2014) and used the group-information guided ICA algorithm to back-reconstruct the 15 corresponding independent network components and their time-courses. Time-varying functional connectivity is represented by estimating 507 dynamic functional network connectivity (dFNC) matrices per subject using the sliding window method (window length: 43.2 s). Subject dFNCs were assigned to 1 of 4 discrete connectivity states by k-means clustering. Finally, we performed two-sample t-tests to identify group differences of dFNC and dFNC-specific measures (level of significance: $p<0.05$, FDR-corrected).
Results: The resulting connectivity states and altered dFNCs are illustrated in Fig. 1. Stroke patients dwelled significantly shorter in State 2 & 3 and showed a trend for shorter fraction times in State 2 that featured specifically weak connectivity within the sensorimotor domain. Furthermore, stroke patients exhibited significantly more frequent transitions to other states.
Conclusions: In sum, the time-varying analysis revealed substantial significant alterations in dwell times and transitions between the four connectivity states when contrasting acute stroke patients with minor motor symptoms and healthy controls. These may reflect the brain's acute reaction to focal damage.

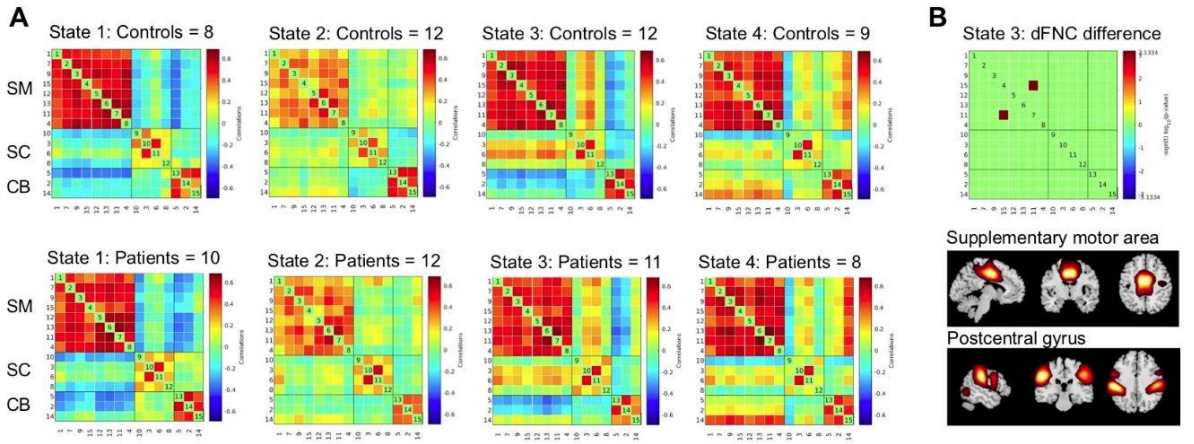


Figure 1. A. Illustration of the four discrete connectivity states and number of subjects visiting the state as resulting from the time-varying functional network connectivity analysis. Correlation values are given as z-scored Pearson correlations, red colors indicate strong positive values, blue strong negative ones (regressed out covariates: age, gender, mean framewise rotation and translation). State 1: Strong positive within domain connectivities, negative connectivities between domains. State 2: Weak positive connectivity within the sensorimotor domain. State 3: Strong positive within domain connectivities, positive connectivity between sensorimotor and subcortical domains, negative connectivity between the sensorimotor, subcortical and cerebellar domains. Qualitatively greatest similarity to the static connectivity matrix (not shown). State 4: Strong positive within domain connectivities, positive connectivity between sensorimotor and cerebellar domains, both weakly positive and negative connectivities between the sensorimotor and subcortical domains. Abbreviations: SM: Sensorimotor components. SC: Subcortical components. CB: Cerebellar components. **B.** FDR-corrected significant connectivity difference in State 3: Healthy controls exhibited a stronger connectivity between the supplementary motor area (SMA) and postcentral gyrus. Connectivity between these areas was significantly positively correlated with motor function in patients. Please note the right-sided lesions were flipped to the left hemisphere.

Allen, E.A., Damaraju, E., Plis, S.M., Erhardt, E.B., Eichele, T. and Calhoun, V.D., 2014. Tracking whole-brain connectivity dynamics in the resting state. *Cerebral cortex*, *24*(3), pp.663-676.

Disclosures: A.K. Bonkhoff: None. **F.A. Espinoza:** None. **H. Gazula:** None. **L. Hensel:** None. **A. Rehme:** None. **L. Volz:** None. **G.R. Fink:** None. **V. Calhoun:** None. **C. Grefkes:** None.

Poster

216. Stroke and Ischemia II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 216.08/G10

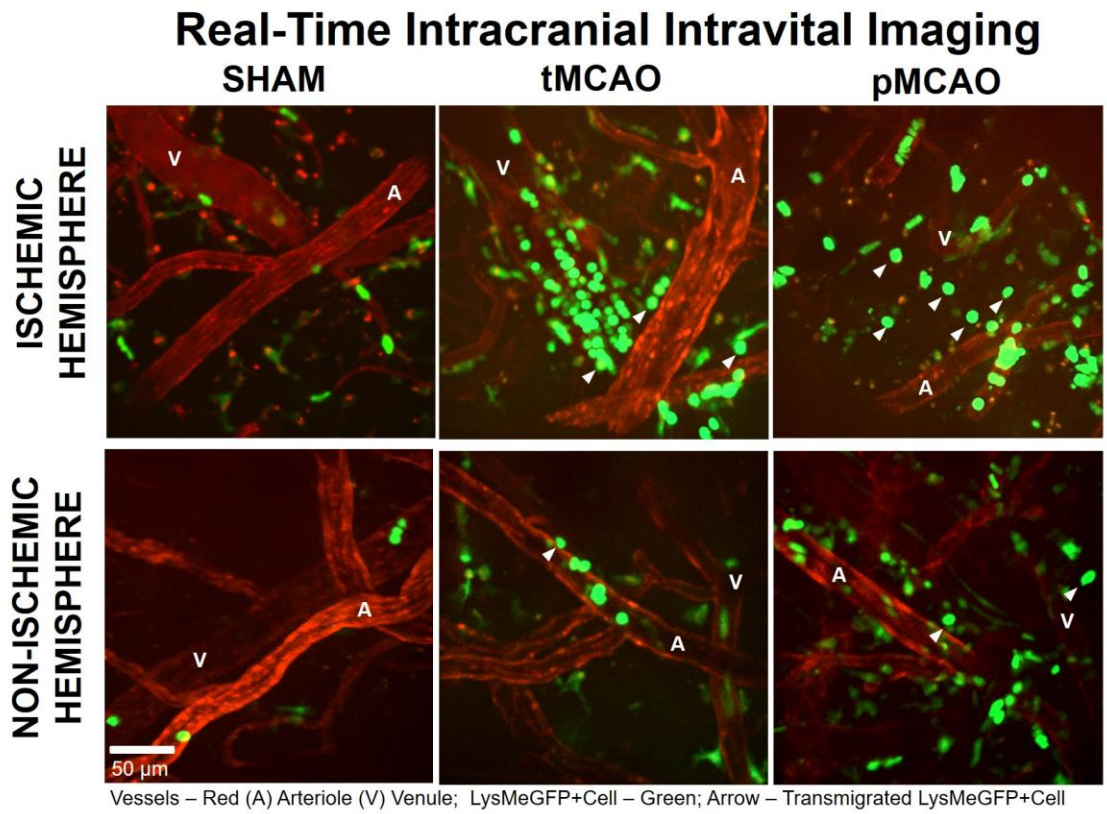
Topic: C.09.Stroke

Support: R01HL064774
R01HL046849
R25NS070695

Title: Intravital microscopy reveals leukocyte recruitment to the cerebrovasculature in both the ischemic and non-ischemic hemispheres in a mouse stroke model

Authors: *A. BATRA[1], N. A. NADKARNI[1], W. A. MULLER[2], D. P. SULLIVAN[2];
[1]Neurol., [2]Pathology, Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: Background: Current treatment for ischemic stroke relies on early restoration of blood flow; however, injury persists despite successful reperfusion. The innate immune response plays a pivotal role in reperfusion injury, but the mechanisms and time-course of leukocyte recruitment to the cerebrovasculature (CBV) during ischemic stroke remain unclear. We sought to characterize the real-time leukocyte response at the CBV in a mouse stroke model with and without reperfusion.
Methods: FVB LysM-eGFP+ mice (10-12 weeks, 25-30g) underwent 60 min. of transient middle cerebral artery occlusion (tMCAO). Real-time spinning-disc confocal intravital microscopy (IVM) of the CBV was performed at 24 hours following reperfusion via an intracranial window in both ischemic and non-ischemic hemispheres and compared to mice undergoing permanent MCAO (pMCAO), sham surgery, and intracranial window placement alone. Leukocyte recruitment (adhesion, rolling and extravasation) were quantified *in vivo* using IVM. Infarct size was confirmed through 2,3,5-triphenyltetrazolium chloride staining post-mortem.
Results: A marked increase in leukocyte recruitment was observed in pMCAO compared to mice undergoing tMCAO at 24 hours. Stroke size in pMCAO mice was more than double that in tMCAO mice. Non-ischemic hemispheres demonstrated increased leukocyte rolling and adhesion, but not extravasation, in the pMCAO group and to a lesser degree in the tMCAO group. Both control groups showed negligible leukocyte recruitment.
Conclusions: Despite reduction in infarct size, a significant leukocyte response persists after reperfusion following tMCAO. Interestingly, this response is present both at the ischemic and the non-ischemic CBV. Acute leukocyte recruitment observed on the non-ischemic hemisphere suggests that ischemic stroke triggers a global inflammatory response. Further reducing inflammation through immunomodulatory therapy in tandem with reperfusion therapy may not only reduce stroke volume but potentially mitigate inflammation mediated secondary neuronal damage.



Vessels – Red (A) Arteriole (V) Venule; LysMeGFP+Cell – Green; Arrow – Transmigrated LysMeGFP+Cell

Disclosures: A. Batra: None. **N.A. Nadkarni:** None. **W.A. Muller:** None. **D.P. Sullivan:** None.

Poster

216. Stroke and Ischemia II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 216.09/G11

Topic: C.09.Stroke

Support: Vascular Dementia Research Foundation
Synergy Excelence Cluster Munich (SyNergy)
ERA-Net Neuron 01EW1501A
Solorz-Zak Research Foundation

Title: vDISCO-clearing- and omics-analysis of skull meninges connection-facilitated trafficking of immune cells after cerebral ischemic stroke in mice

Authors: *B. FÖRSTERA[1], H. MAI[1], Z. I. KOLABAS[1], K. STANIC[1], Z. RONG[1], F. HELLAL[1], M. MOLBAY[1], H. S. BHATIA[1], M. STERR[2], M. LOTFOLLAHI[3], A.-D.

BRUNNER[4], S. ZHAO[1], M. I. TODOROV[1], R. CAI[1], C. PAN[1], H. LICKERT[2,5], F. THEIS[3,6], M. MANN[4,7], A. ERTURK[1];
[1]Inst. for Stroke- & Dementia-Research (ISD), Klinikum der Univ. München (KUM), Munich, Germany; [2]Inst. of Diabetes and Regeneration Res., [3]Inst. of Computat. Biol. (ICB), Helmholtz Zentrum München, Munich, Germany; [4]Proteomics and Signal Transduction, Max Planck Inst. of Biochem., Martinsried near Munich, Germany; [5]Fac. of Med., [6]Fac. of Mathematics, Tech. Univ. Munich (TUM), Munich, Germany; [7]Clin. Proteomics, Univ. of Copenhagen (UCPH), Copenhagen, Denmark

Abstract: Cerebral ischemic stroke is one of the world's leading causes of death and disability, yet therapeutic options are limited. It is characterized by an invasion of systemic immune cells into the central nervous system. Understanding the process of neuro-inflammation holds the promise of developing new, disease modifying treatments. Using vDISCO-labelling and clearing, we recently described short vascular connections between the skull and the brain (Cai et al. Nat. Neurosci. 2019). Moreover, it has been reported that invading granulocytes are preferentially recruited from the skull bone marrow after stroke (Herisson et al. Nat. Neurosci. 2018). Accordingly, these direct skull meninges connections described by us and others present a potential shortcut for immune cells to infiltrate the brain after injury. Here we present new data acquired via whole body light-sheet imaging after vDISCO, proteomics and single cell transcriptomics after transient middle cerebral artery occlusion (fMCAO), to elucidate the roll of skull meninges connections in neuro-inflammation after cerebral ischemic stroke in mice. Single cell RNAseq analysis confirmed that neutrophils are the most abundant type of resident immune cells in the bone marrow. While we detected similar cell populations in the bone marrow of control and stroke animals, we found that several genes are differentially expressed in the same cell type clusters. To obtain more insight about the molecular identities of these cells, we performed proteomics using the Orbitrap Mass Spec platform. We found that the proteome of the bone marrow is significantly different after stroke compared to the physiological state. Furthermore, we show that the number of LysM positive neutrophils and macrophages drastically increases in the bone marrow as well as the skull meninges connections after cerebral ischemic stroke. Taken together, these findings suggest that the bone marrow and the skull meninges connections play a major role in the neuro-inflammatory process after cerebral ischemic stroke, highlighting the cells in this area as a potential target for novel diagnostic and therapeutic approaches.

Disclosures: B. Förstera: None. **H. Mai:** None. **Z.I. Kolabas:** None. **K. Stanic:** None. **Z. Rong:** None. **F. Hellal:** None. **M. Molbay:** None. **H.S. Bhatia:** None. **M. Sterr:** None. **M. Lotfollahi:** None. **A. Brunner:** None. **S. Zhao:** None. **M.I. Todorov:** None. **R. Cai:** None. **C. Pan:** None. **H. Lickert:** None. **F. Theis:** None. **M. Mann:** None. **A. Erturk:** None.

Poster

216. Stroke and Ischemia II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 216.10/G12

Topic: C.09.Stroke

Support: NIH R01 HL046849
NIH R37 HL064774
NIH R25 NS070695

Title: Ischemic stroke induces non-homogenous inflammatory leukocyte response within the ischemic core and penumbra

Authors: *D. P. SULLIVAN[1], A. BATRA[2], N. A. NADKARNI[2], W. A. MULLER[1];
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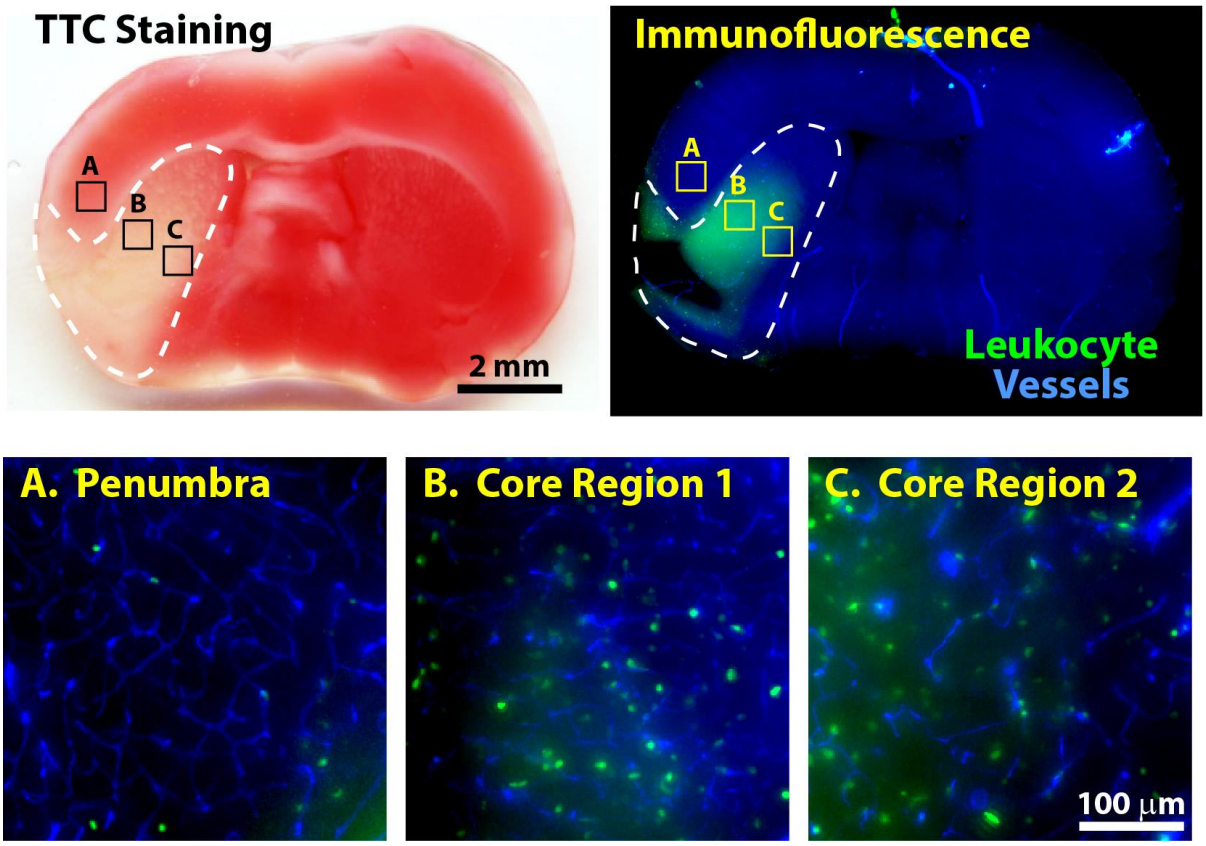
Abstract: Stroke is a leading cause of death and disability in the US. All current therapies are predicated on reperfusion, with no therapies directed at the inflammatory response. Several promising interventions aimed at modulating inflammation by disrupting leukocyte infiltration failed in human trials, despite positive results in mice. One explanation for this disconnect is an incomplete understanding of the spatio-temporal recruitment and infiltration of leukocytes. Here we investigate the evolution of the inflammatory response in a mouse stroke model, the transient middle cerebral artery occlusion (tMCAO). We used wide-field and confocal immunofluorescence microscopy and a variety of markers to examine brain sections at several time points post reperfusion to examine the exact nature and location of the invading myelomonocytic population.

Our findings suggest that majority of recruited leukocytes escape the perivascular compartment, but that this extravasation follows an unusual time course. More specifically, leukocyte extravasation and accumulation in the core infarcted tissue appears to be unexpectedly delayed, suggesting that factors unique to the neurovascular unit are modulating the inflammatory response. We also observed that triphenyl tetrazolium chloride (TTC) staining, a common indicator of infarcted tissue, does not correlate with the degree or location of leukocyte infiltration. Dramatic heterogeneity in the inflammatory infiltrate was observed across the ischemic core, but also in the surrounding penumbra and cortical surface. This indicates that although TTC staining is a poor prognosticator for the regions that are susceptible to inflammation.

Taken together our findings suggest that current methodologies insufficiently describe the inflammatory milieu after ischemic stroke and that careful reconsideration is warranted. A better understanding of the precise spatio-temporal infiltration of inflammatory cells could help inform the next generation of therapeutic interventions targeting the inflammatory response.



Disclosures: **D.P. Sullivan:** None. **A. Batra:** None. **N.A. Nadkarni:** None. **W.A. Muller:** None.

Poster

216. Stroke and Ischemia II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 216.11/G13

Topic: C.09.Stroke

Support: RSF Grant 18-15-00229
State Assignment Project 18.2583.2017/4.6

Title: Macromolecular proton fraction parameter as a marker of myelin recovery in the model of local ischemia in rats

Authors: ***M. KHODANOVICH**[1], M. KUDABAEVA[1], V. GLAZACHEVA[1], I. GUBSKIY[2], D. NAMESTNIKOVA[2], V. YARNYKH[3];

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Abstract: *Introduction:* In addition to neuronal death, ischemic stroke causes extensive demyelination in the site of lesion. Enhancement of post-ischemic endogenous neurogenesis and oligodendrogenesis is considered as potential target for brain recovery. A recently developed quantitative MRI method, fast macromolecular proton fraction (MPF) mapping, has been used to quantify myelination. This method showed a promise as a biomarker of myelin in human and animal studies, including the model of stroke. *Purpose:* The study aimed to estimate MPF mapping as a non-invasive biomarker of recovery after stroke. *Methods:* For modeling local ischemia, male Wistar rats (n=6) underwent surgery with transient occlusion of the middle cerebral artery (MCAO) followed by reperfusion. MPF maps were obtained using the single-point synthetic reference method on a Bruker BioSpec 11.7T small-animal MRI scanner. MPF mapping was performed before and at days 3, 5, 7, 14, 21, 31, 42 and 56 after the surgery; histological processing included time points 7, 21 and 56 days after surgery. Brain sections were labeled with GFAP (astrocytes), MBP (myelin), LFB (myelin), NeuN (mature neurons), DCX (young new neurons) and NG2 (oligodendrocyte precursors). *Results:* Absolute values of MPF in the ischemic core at days 3-7 decreased to 5-7% from the baseline of approximately 10%. At days 7-56 MPF values showed opposite changes in two distinctly visible zones of the ischemic core. MPF values further decreased to 2-5% in the demyelination zone (DZ) and increased almost up to the baseline in the remyelination zone (RZ). LFB and MBP labeling showed similar division into the zones of demyelination and remyelination, which corresponded to those in MPF maps, at days 21 and 56. Myelin fibers in the RZ were substantially reorganized. Reactive astrocytes formed glial scar around the DZ at day 56. Neuronal loss was 94% in the DZ and 25% in the RZ. The density of neuroblasts and oligodendrocyte precursors was increased throughout almost the whole lesioned striatum from the SVZ to the DZ and RZ. The most prominent increase was observed in the DZ.*Conclusions:* Longitudinal observations showed active processes of neurogenesis and oligodendrogenesis after stroke in the MCAO model. The study reveals the feasibility of using MPF as a specific biomarker of demyelination and remyelination, which has potential clinical significance in rehabilitation after stroke.*Acknowledgements:* Russian Science Foundation, project #18-15-00229, State Assignment Project #18.2583.2017/4.6.

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Poster

216. Stroke and Ischemia II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 216.12/G14

Topic: C.09.Stroke

Support: ERDF (No. CZ.02.1.01/0.0/0.0/16_013/0001775)
OPPK BrainView CZ.2.16/3.1.00/21544
Czech-BioImaging large RI project (LM2015062 funded by MEYS CR)
SGS18/137/OHK3/2T/13
15-33115A

Title: MRI maturation of brain ischemic tissue in human patients after stroke

Authors: D. KALA[1,2], A. POSUSTA[3], V. SULC[4], P. JANSKY[4], A. TOMEK[4], P. MARUSIC[4], P. JIRUSKA[3], J. OTAHAL[5];
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Abstract: Cerebral ischemia is one of the most common cerebrovascular disease. Ischemic processes are traditionally detected by MRI scanning using FLAIR (fluid attenuation inversion recovery) or DWI (diffusion weighted imaging) sequence. Lesion identification was well described in early and acute phase of ischemia (first hours – 2 days after insult) but less is known about changes on FLAIR and DWI in later phase (2-12 days) in human. Aim of this study is to affected brain regions based on FLAIR and DWI MRI and describe their changes during maturation of ischemic tissue.
We have analyzed 11 patients after cerebral ischemia. Patients underwent MRI scanning with experimental protocol consisting FLAIR, DWI and T1 weighted sequence in two time points; in acute phase of ischemia (2-5 days after ischemic insult) and subacute phase (7-12 days). 5 regions of interest (ROI) were defined and masked from MRI using thresholding of pathological hyperintensities from healthy tissue. ROIs were defined as hyperintensity on FLAIR (FLAIR+), on DWI (DWI+) and its combinations FLAIR+DWI+, FLAIR+DWI- and FLAIR-DWI+. Volume of every ROI was calculated in both timepoints.
We have analyzed changes in volumes of individual ROIs between acute and subacute phase of ischemia. We have observed significant ($p=0.04$) reduction (25% in median) of volume in DWI+ ROI, whereas other ROIs were not significantly changed during tissue maturation.
Reduction of DWI+ ROI can be understood as manifestation of retraction of cytotoxic edema between acute and subacute phase. However, vasogenic edema visible on FLAIR+ persisted unchanged.

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Poster

216. Stroke and Ischemia II

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Program #/Poster #: 216.13/G15

Topic: C.08. Ischemia

Support: NIH Grant NS084744
NIH Grant HD080429
NIH Grant NS095064
NIH Grant NS106592

Title: A modified recombinant tissue plasminogen activator-responding photothrombotic stroke model

Authors: *Y.-M. KUO[1,2], Y.-Y. SUN[1], H.-R. CHEN[1], C.-Y. KUAN[1];
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Abstract: Photodynamical dye-based stroke model (e.g. Rose Bengal [RB]/photothrombosis) has several attractive features, including simple surgical procedures, consistent infarct size, and low mortality rate. Yet, it poorly responds to recombinant tissue plasminogen activator (rtPA)-based lytic therapy, presumably due to fibrin-poor clots, and making it less suitable for studying reperfusion injury or improving lytic therapy. Hence, the goal of this study is to devise a modified, rtPA-responding photothrombotic stroke model. We hypothesize the addition of thrombin to RB dye in photothrombosis will increase the fibrin component in clots, making it more susceptible to rtPA-thrombolysis. Based on the standard RB/photothrombotic stroke procedures, we mixed a subthreshold level of 80 U/kg bovine thrombin with 50 mg/kg RB and injected the mixture intravenously to mice, followed by laser illumination (5 mW, 543.5 nm) at the proximal branching point of the middle cerebral artery (MCA) for 20 min to create a thrombin-Rose Bengal (TRB)/photothrombotic stroke model. We compared the clot composition, effects of rtPA treatment on cerebral blood flow reperfusion, and the infarct size following rtPA treatment initiated at 30, 60, or 120 min after photothrombosis. Here we show that, 1) the addition of thrombin did not increase mortality rate or infarct size, when compared to standard RB/photothrombosis. 2) Immunostaining revealed fibrin mixed with platelets in TRB/photothrombosis-induced clots. 3) Laser speckle contrast imaging indicated that intravenous infusion of rtPA (10 mg/kg) at 30 min after TRB/photothrombosis restored blood flow in the MCA-supplied territory, but the same treatment caused little reperfusion in RB/photothrombosis. Similarly, the infarct size was reduced after rtPA treatment in TRB/photothrombosis, but not in RB/photothrombosis ($n > 8$ in each). 4) The therapeutic window of rtPA-treatment is at least 120 min in TRB/photothrombosis ($n > 10$ in each). Our

TRB/photothrombosis model results in fibrin/platelet composite clot, making it far more responsive to rtPA thrombolytic treatment. This modified, rtPA-responding thrombotic stroke model may have broad applications in experimental stroke research.

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Poster

216. Stroke and Ischemia II

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Program #/Poster #: 216.14/G16

Topic: C.08. Ischemia

Support: NIH grant 5R01ES024936-05
NIH grant 5R01NS100459-03

Title: BBB breakdown leads to white matter changes in the mouse model of bilateral carotid artery stenosis

Authors: *M. T. HUUSKONEN, Q. LIU, A. MONTAGNE, W. J. MACK, B. V. ZLOKOVIC; Keck Sch. of Med. of the USC, Los Angeles, CA

Abstract: Based on clinical studies carotid artery stenosis is strongly connected to cognitive decline. In the mouse model of this condition two MRI compatible micro-coils are inserted around the carotid arteries of a mouse which leads to chronic cerebral hypoperfusion. Interestingly, this model has been shown to produce relatively specific structural and functional white matter damage without the involvement of gray matter areas. It has been suggested that the white matter damage in this model is mainly caused by neuroinflammatory cascades initiated by hypoperfusion. However, the role of BBB damage on white matter changes in this model remains unclear. Here, we have used longitudinal multiparametric MRI protocol 1, 3, 7 and 30 days after the operation to visualize changes in anatomical structures (T2w), angioarchitecture (TOF angiography), BBB permeability (DCE-MRI) and cerebral blood flow (DSC-MRI) *in vivo*. All imaging was performed with combined 7T PET/MRI scanner (MR solutions) and using gadolinium-based contrast agent. Analysis of DCE and DSC MRI data was performed using Rocketship software. Based on our results bilateral carotid artery stenosis leads to early BBB opening which is later alleviated by rearrangement of angioarchitecture and simultaneous increase in CBF. These changes precede white matter rarefaction, which is seen approximately 1 month after the initiation of hypoperfusion. In conclusion, early BBB breakdown contributes to the characteristic white matter damage in the mouse BCAS model.

Disclosures: M.T. Huuskonen: None. **Q. Liu:** None. **A. Montagne:** None. **W.J. Mack:** None. **B.V. Zlokovic:** None.

Poster

216. Stroke and Ischemia II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 216.15/G17

Topic: C.08. Ischemia

Title: Heme degradation products are involved in the pathogenesis of early- and late-onset vasospasm following subarachnoid hemorrhage

Authors: *A. JOERK[1,2], M. RITTER[7], D. FREITAG[3], K.-H. HERRMANN[4], R. A. SEIDEL[7,5], N. LANGGUTH[1], A. SCHAEFGEN[1], M. RITTER[1,6], S. C. SCHRÖDER[1,6], D. SCHULZE[7], G. POHNERT[7], M. WESTERHAUSEN[7], J. WALTER[3], R. KALFF[3], J. R. REICHENBACH[4], P. RUTH[8], O. W. WITTE[1], K. HOLTHOFF[1];
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Abstract: BACKGROUND: Vasospasm-induced delayed cerebral ischemia (DCI) is the most common determinant of mortality and unfavorable prognosis in patients suffer from subarachnoid hemorrhage (SAH). Actually, guideline-based recommendations and experimental approaches to vasospasm treatment failed to improve the clinical outcome. We hypothesize that heme degradation products (HDPs), originating from the hematoma surrounding the ruptured aneurysm, are involved in vasospasm pathogenesis depending on BK potassium channel expression. HDPs, comprising propentdyopents (PDPs) and bilirubin oxidation products (BOXes), are present in the cerebrospinal fluid of SAH patients and induced a vasoconstrictive effect on mouse cerebral microcirculation.
METHODS: The short-onset vasoactivity of HDPs was investigated on acute mouse brain slices, illuminated with DIC microscopy. To study late-onset effects on cerebral perfusion, SAH was experimentally induced in adult wildtype and BK channel deficient mice by the subarachnoid injection of autologous blood or PDP and BOX isomers into the cisterna magna, followed by temporally high-resolution MR perfusion imaging series at 9.4T. Subsequent to MRI, vessel wall morphology of cerebral arteries was analyzed in PFA-fixed brain slices stained with anti-smooth muscle actin and Ki-67.
RESULTS: In acute brain slices, the vasoconstrictive effect of PDPs decreased in aged mice. Furthermore, PDP isomers failed to induce arterial vasoconstriction in smooth muscle-specific BK knockout mice. In intact animals, the subarachnoid injection of autologous blood or PDPs induced a reversible cortical perfusion delay in wildtype mice on postinterventional days 3 and 7 in comparison to control. This perfusion disturbance was absent in PDP-treated BK knockout

mice. In contrast, subarachnoidally injected BOX isomers failed to induce cerebral perfusion deficits. These data were complemented by immunofluorescence microscopy where blood and HDPs induced an increase of smooth muscle wall thickness in cerebral arteries until the postinterventional day 14.
CONCLUSION: Besides the short-onset vasoactivity, our data demonstrate a long-term and age-dependent effect of PDPs on cerebral perfusion which correlates with the delayed manifestation of vasospasms in SAH patients. These findings may promote novel strategies for vasospasm treatment.

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Poster

216. Stroke and Ischemia II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 216.16/G18

Topic: C.08. Ischemia

Support: NS090904

Title: A white matter stroke model induced by injection of a vasoconstrictor in internal capsule

Authors: *Y. WANG[1], M. T. HUUSKONEN[1], A. MONTAGNE[1], B. V. ZLOKOVIC[2]; [2]Zilkha Neurogenetic Inst., [1]Keck Sch. of Med. of the Univ. of Southern California, Los Angeles, CA

Abstract: White matter (WM) injury is common in stroke patients. The most commonly affected WM areas are corpus callosum (CC), periventricular WM (PVWM), and the posterior limb of the internal capsule (PLIC). Infarcts of the internal capsule (IC) have been linked to motor impairment and poor prognosis in stroke patients. Subcortical WM stroke models have not been extensively studied and optimization of clinically relevant models is needed. In this study, we developed a new mouse model of lacunar infarction within the IC using stereotaxic injections of a vasoconstrictor N5-(1-iminoethyl)-L-ornithine (L-Nio) dihydrochloride (Calbiochem). The ischemic injury was verified by Cresyl Violet staining and T2-weighted high-field magnetic resonance imaging (MRI) 24 hours after L-Nio. In addition, behavioral tests (foot-fault test, rotarod test, and nesting test) were performed 24 hours, 1 week, and 4 weeks following L-Nio. We found that intracerebral injections of L-Nio produced a 100% success rate in inducing a lesion within the IC and mice develop sensorimotor function deficits. These results demonstrate

that our new IC stroke model is, not only clinically relevant, but is reliable and reproducible and can be used to study the etiology of WM infarcts.

Disclosures: Y. Wang: None. **M.T. Huuskonen:** None. **A. Montagne:** None. **B.V. Zlokovic:** None.

Poster

216. Stroke and Ischemia II

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Program #/Poster #: 216.17/G19

Topic: C.08. Ischemia

Support: NIH grant R01NS046400
Florida Department of Health
São Paulo Research Foundation

Title: Role of PGE_2 EP1 receptor antagonist on stroke outcomes in Alzheimer's disease mouse models

Authors: F. R. MENDES[1], J. L. LECLERC[2], L. LIU[3], P. K. KAMAT[3], A. NAZIRIPOUR[3], D. HERNANDEZ[3], C. LI[3], ***A. S. AHMAD**[3], S. DORE[3,4];
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Abstract: Background: AD is the most common memory disorder and its prevalence is expected to triple by the year 2050. Neuroinflammation is recognized as an important player in the pathogenesis of AD. One of the most recognized pathways in mediating the neuroinflammation is of prostaglandin E_2 (PGE_2) EP1 receptor pathway. Role of PGE_2 EP1 receptor in AD mouse models following stroke has not been studied. In this study, we examined the efficacy of a selective EP1 antagonist, ONO-8713, on lesion volumes and behavioral indexes in AD mouse models after stroke. Methods: Two cohorts of transgenic, APP/PS1 and 3xTg, and wildtype (WT) mice were subjected to permanent distal middle cerebral artery occlusion (pdMCAO) and sham surgeries. EP1 antagonist ONO-8713 or vehicle was then administered to analyze its effect on anatomical and functional outcomes. Results: The functional outcomes were significantly deteriorated in the APP/PS1 and the 3xTg mice after stroke. Interestingly, the ONO-8713-treated groups of WT mice performed significantly ($p<0.001$) better in open field test than the respective groups of 3xTg mice. For the passive avoidance test, APP/PS1+ONO-8713 mice exhibited significant ($p<0.05$) difference in retention as compared with the vehicle group; whereas, there

was a significant difference in acquisition between WT+ONO-8713 and 3xTg+ONO-8713 ($p<0.05$). There was a significantly lower tissue injury in APP/PS1+Veh mice when compared to the APP/PS1+ONO-8713 mice ($p<0.02$). Percent tissue injury was significantly higher in APP/PS1+ONO-8713 mice when compared to WT+ONO-8713 mice ($p<0.05$). Similarly, in the 3xTg cohort, percent tissue injury and percent tissue loss were significantly higher in 3xTg+ONO-8713 mice than in WT+ONO-8713 mice ($p<0.02$). Conclusion: The EP1 receptor antagonist ONO-8713 shows some beneficial effects on functional and anatomical outcomes after stroke in APP/PS1 and 3xTg mice models of AD; though, the effects were not significant in all outcomes investigated. Further studies are needed to understand the role and optimal timing of EP1 receptor blockade in the context of AD etiopathology.

Disclosures: F.R. Mendes: None. **J.L. Leclerc:** None. **L. Liu:** None. **P.K. Kamat:** None. **A. Naziripour:** None. **D. Hernandez:** None. **C. Li:** None. **A.S. Ahmad:** None. **S. Dore:** None.

Poster

216. Stroke and Ischemia II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 216.18/G20

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH Grant NS065808

Title: Long-term persistence of AAV DNA is compromised in neuroglial progenitors by cell proliferation

Authors: *G. J. HELLER[1], D. NGUYEN[2], M. S. SANDS[3], E. BONGARZONE[2]; [1]Neurosci., [2]Anat. and Cell Biol., Univ. of Illinois, Chicago, Chicago, IL; [3]Genet., Washington Univ., St Louis, MO

Abstract: Adeno-associated viruses (AAVs) are rapidly becoming the gold standard of the gene therapy world, they are the subject of investigation of a multitude of preclinical investigations and clinical trials. AAV gene therapy seems particularly effective at treating monogenic neurodegenerative diseases such as several lysosomal storage diseases. AAVs are low/no immunogenic, appear to have low/none toxicity, and in general, are effective for maintaining significant long-term expression of therapeutic genes. This is particularly true for diseases primarily affecting post-mitotic cells such as neurons or muscle cells. On the other hand, much less is known on the long-term therapeutic effectivity of AAVs in diseases affecting actively dividing cells, such as oligodendrocyte progenitors or astrocytes. Because AAV inserts its DNA payload into a cell largely as non-replicating episomes, we speculate that as cells divide, the average transgene DNA per cell will decrease, leading to reductions in therapeutic levels of AAV-DNA.

To challenge our hypothesis, we first utilized an *in vitro* model for Krabbe's disease (KD), a lysosomal storage disease caused by the deficiency of galactosylceramidase (GALC). Neural progenitors were isolated from the subventricular zone of the twitcher mouse, a natural KD model and transduced with AAV-GALC at 5000 MOI. After transduction, cultures were continually grown with samples analyzed every 4 days for 20 days. AAV DNA and GALC activity were measured in comparison with untreated Twitcher and control cells. There was a rapid decrease in average AAV-GALC DNA and GALC enzyme activity per cell within the first 12 days, inversely proportional to the increase in cells. Secondly, we intracranially injected one-day old control pups with AAV-GFP at a dose of 1e8 viral particles per animal. Mice were analyzed for GFP expression, at 1 week (considered the baseline of transduction), 1 month, and 2 months. There was a gradual decline in the GFP expression as the mice aged. Decrease was observed in dividing glia but not in post-mitotic neurons, in support of our hypothesis of AAV genome dilution in proliferating glia over time. Our results underscore the need to consider this long-term dilution effect in gene therapy targeting proliferating cells in the CNS.

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Poster

216. Stroke and Ischemia II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 216.19/G21

Topic: C.08. Ischemia

Support: Grants-in-Aid for Scientific Research (C) 26461320 from the Japan Society for the Promotion of Science
AMED Japan Regenerative Medicine Project (Grant 15bk0104012h003)

Title: Regeneration associated cells transplantation reduce the neural death in stroke mice by intravenous injection

Authors: *T. NAKAYAMA[1], E. NAGATA[1], H. MASUDA[2], T. ASAHARA[3], S. TAKIZAWA[1];
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Abstract: Background: Endothelial Progenitor Cell (EPC) was reported to enhance repairing and regenerating neurovascular units. So far many papers have reported repairing and regenerating experiments using EPCs derived from bone marrow, spleen, or peripheral blood. We got higher grade quality EPCs, Regeneration-associated cells (RACs), using a novel colony assay system which we have developed (Masuda H, et al, 2011). Already we reported the reduction of

ischemic stroke volume, anti-inflammatory effect, and vasculogenesis in stroke mice by interatrial injection of RACs (Taira N, et al, 2019)
Materials and methods: We made 35 ischemic stroke model mice (10 weeks male C57BL/6 mice) with permanent middle cerebral artery occlusion (MCAO). We injected PBS as control (n=15), 10^4 cells/μL RACs (n=10), and 10^5 cells/μL RACs (n=10) into tail vein at 3 days after MCAO. At 1 weeks after MCAO, we took the brains and investigated time-lapse physiological parameters including cerebral blood flow and immunohistochemistry against some anti-vasculogenetic factor antibodies.
Results: In the stroke model mice at RACs injections (10^5 cells/μL) after MCAO, the stroke volume was decreased. Some anti-inflammatory factors tended to increase in RACs injection mice.
Conclusions: Those results indicate that the RACs transplantation has similar effects by intravenous as well as by intraarterial injection; however, the more RACs needed for intravenous than intraarterial injection in mice.

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Poster

216. Stroke and Ischemia II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 216.20/G22

Topic: C.08. Ischemia

Title: Focused ultrasound-induced intracerebral hemorrhage at two distinct pressures causes differing outcomes in mice

Authors: *C. M. D. COLLIER[1], H. ZHANG[2], E. E. KONOFAGOU[2], C. M. TROY[3]; [1]Neurobio. and Behavior, [2]Engin., Columbia Univ., New York, NY; [3]Dept Pathol & Neurol, Columbia Univ. Medl Ctr., New York, NY

Abstract: Intracranial bleeding is a phenomenon that spans a wide array of clinical outcomes from asymptomatic cerebral microbleeds to debilitating hemorrhagic stroke. Much of our understanding of these conditions has relied on the study of animal models that recapitulate portions of the intracerebral hemorrhage pathophysiology. We have developed a pressure-based mouse model of intracerebral hemorrhage using Focused Ultrasound in combination with circulating microbubbles. Focused Ultrasound is a well-established method for transient blood-brain-barrier opening and is an area of investigation for various therapeutic applications. When performed using high pressures and intensities this technique is noted to cause vessel rupture and histological damage. We have employed this technique at two pressures, both above the threshold to induce hemorrhage, resulting in distinct outcomes. Mice, following induction of

intracerebral hemorrhage, underwent behavioral analysis including vibrissae-evoked forelimb placement and the corner turn test. At the lower of the two pressures, mice appear asymptomatic but at the higher pressure, behavioral analysis demonstrates neurological deficits. Following sacrifice, the levels of inflammation and cell death within the brain are also assessed. Our results demonstrate that the injury produced by Focused Ultrasound-Induced Intracerebral Hemorrhage (FUS-ICH) is modifiable by adjusting the pressure. The distinct outcomes observed in this study demonstrates the potential applicability of the FUS-ICH model to a range of conditions involving intracranial bleeding.

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Poster

216. Stroke and Ischemia II

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Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 216.21/G23

Topic: C.08. Ischemia

Support: 1R21NS108386-01A1

Title: Comparative striatal DA neurotransmission characteristics across cardiac arrest models and severities

Authors: *D. FINE[1,5], J. STEZOSKI[5,2], R. HARUN[1], R. LEAK[6], P. M. KOCHANEK[5,1], T. DRABEK[5,3], A. K. WAGNER[5,1,4];
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Abstract: Cardiac arrest (CA) survival rates have improved with modern resuscitation techniques and clinical care, but many survivors experience functional impairments after their hypoxic-ischemic brain injury (HIBI). Reduced arousal and behavioral/cognitive symptoms commonly observed after CA indicate striatal dopamine (DA) dysfunction. As such, DA agonists are often used to treat these populations. However, the molecular underpinnings of these neurological sequelae, their differences across different CA injuries and severities, and their respective impact on DA neurotransmission are largely unknown. We hypothesized ventricular fibrillation (VF)-CA and asphyxia CA (ACA) would produce different effects on DA neurotransmission. Adult male Sprague-Dawley rats (n=25) were anesthetized and underwent 5-minute VF-CA, 6-minute VF-CA, 5-minute ACA, or sham surgery, which replicates CA procedures with the exception of the insult Fast-scan cyclic voltammetry was used with median forebrain bundle electrical stimulations (60Hz, 10s) to characterize presynaptic DA

neurotransmission in the dorsal striatum (D-STR) 14-days after CA/sham surgery. ACA animals tended to have lower maximum evoked D-STR DA overflow versus VF-CA($p=0.06$). ACA animals also exhibited lower DA release rate ($p<0.05$), overall DA released ($p<0.01$), and maximal reuptake velocity (Vmax)($p<0.05$) in the D-STR compared to VF-CA. Further, 6-minute VF-CA animals exhibited increased maximum evoked DA overflow in the D-STR ($p=0.055$) as well as the nucleus accumbens (NAc) ($p<0.05$) compared to 5-minute VF-CA animals. 6-minute VF-CA animals also had increased DA overflow at 1s into stimulation ($p<0.05$), total DA released ($p=0.051$), and DA release rate ($p<0.05$) in the D-STR compared to 5-minute VF-CA animals. These data have implications for CA treatment, suggesting precision medicine approaches may require a multimodal approach to evaluating etiology of cardiac-arrest as a variable factor when testing treatments impacting DA neurotransmission across models, thus optimizing recovery and DA therapeutics for survivors of various CA types and severities.

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Poster

216. Stroke and Ischemia II

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Topic: C.08. Ischemia

Support: NST-KIST Postdoctoral Research Fellowship for Young Scientist

Title: Three-dimensional architectural images using the whole-brain staining in chronic cerebral hypoperfusion-induced mice

Authors: *M. HAN[1,2], M.-S. KIM[1,2], J. BANG[1,2], J.-I. KIM[1,2], M. NA[3], S. CHANG[3,4,5], W. JEON[1,2];
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Abstract: Clinical and experimental evidence suggests that chronic cerebral hypoperfusion (CCH) is the common underlying mechanism of cognitive decline processes, like vascular cognitive impairment and Alzheimer's disease. However, three-dimensional whole-brain architecture is poorly understood because of the limited imaging depth. Recently, tissue-clearing methods were developed, which allow three-dimensional visualization. In this study, we investigated if CCH could affect brain structure, especially, blood vessels, neurons, and

astrocytes. Adult C57BL/6J and Thy1-ChR2-YFP mice underwent unilateral common carotid artery occlusion (UCCAo) to induce CCH. Their brains were isolated for Clear Lipid-exchanged Acrylamide-hybridized Rigid Imaging-compatible Tissue-hYdrogel (CLARITY) processing at various time-points after surgery. Whole-brain immunostaining was performed to label blood vessels, astrocytes, and neurons. Imaging was performed with confocal and light-sheet microscopy; the three-dimensional images were then visualized using Imaris software. The three-dimensional whole-brain image had an imaging depth that was 250 times greater than that of traditional two-dimensional images. Moreover, cellular resolution of the whole-brain image allowed understanding of the neuronal-glial-vascular units, which are essential for the function of the central nervous system. In conclusion, this anatomical information could be useful for understanding the effects of CCH on memory and cognition.

Disclosures: M. Han: None. **M. Kim:** None. **J. Bang:** None. **J. Kim:** None. **M. Na:** None. **S. Chang:** None. **W. Jeon:** None.

Poster

216. Stroke and Ischemia II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 216.23/DP06/G25

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: C.08. Ischemia

Support: NIH Grant K12HD073945
NIH Grant WaNPCR, P51 OD010425
NSF Grant EEC-1028725

Title: A practical method for inducing focal ischemic stroke in non-human primate cortex

Authors: K. KHATEEB[1], Z. YAO[2], D. J. GRIGGS[1], S. SONG[1], V. KHARAZIA[3], R. WANG[1], ***A. YAZDAN-SHAHMORAD**[1];
[2]Bioengineering, [1]Univ. of Washington, Seattle, WA; [3]Ernest Gallo Clin. and Res. Ctr., Emeryville, CA

Abstract: Ischemic stroke is the leading cause of long-term disability among adults in the United States. Despite its prevalence, few therapeutic options exist to treat sensory and motor dysfunctions that arise as a result of stroke. Current stroke studies are mostly limited to rodent models, which have failed to yield positive results in clinical trials. Moreover, non-human primate (NHP) models of ischemic stroke are highly variable in efficacy and require complex surgical skill. We therefore developed a reproducible method for inducing targeted ischemic lesions in cortex of NHPs by utilizing the photothrombotic model, a method previously

developed for rodents. In this model, light illumination of tissue following intravenous (IV) injection of the photosensitive dye Rose Bengal results in the formation of localized thrombi, generating focal ischemic lesions. Here we present a quantitative model to predict the extent of ischemic damage followed by *in vivo* validation of the model in two rhesus macaques. To predict the extent of photothrombotic ischemic damage following light irradiation, we applied McLean's light beam spread function to model the light intensity distribution of a collimated light beam through cortical tissue. We then implemented the photothrombotic technique in two adult male rhesus macaques. Regions of the sensorimotor cortex were illuminated by a cold light source for 30 minutes through 0.5 - 2.0 mm diameter apertures following IV injection of Rose Bengal. To enable *in vivo* monitoring of the extent of ischemic damage, optical coherence tomography angiography (OCTA) images were captured before and 3 hours after illumination. Subsequent histological analysis was performed to further assess lesion sizes. Our predictive quantitative model estimated lesion sizes to roughly correspond with the diameters of the incident light beams. *In vivo* OCTA imaging confirmed the formation of ischemic lesions 3 hours after illumination with lesion diameters consistent with the predictions of our quantitative model. Similarly, histological analysis further validated our model by enabling us to assess other critical lesion characteristics such as depth, wherein lesions encompassed all layers of the cortex. The use of OCTA imaging will enable for the implementation of this technique for chronic experiments to assess the sizes of the ischemic lesions *in vivo*. Our results demonstrated the ability to reliably control and replicate the lesion sizes and locations. This model has the potential to enhance our understanding of local network dynamics within regions of the ischemic penumbra and can be used to develop effective neurorehabilitative strategies.

Disclosures: A. Yazdan-Shahmorad: None. **K. Khateeb:** None. **D.J. Griggs:** None. **Z. Yao:** None. **S. Song:** None. **V. Kharazia:** None. **R. Wang:** None.

Poster

216. Stroke and Ischemia II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 216.24/G26

Topic: C.08. Ischemia

Title: Comparison of behavioural and imaging outcome between different occlusion time in transient cerebral artery occlusion (tMCAO) model in Balb/c mice

Authors: M. DUDEK, T. BRAGGE, A. SHATILLO, ***A. J. NURMI**, D. MISZCZUK; Charles River Discovery, Kuopio, Finland

Abstract: Nearly 90% of stroke cases are ischemic and occur in the region of the middle cerebral artery. Numerous candidate drugs have been tested in clinical trials however, only one pharmacological treatment, tissue plasminogen activator (tPA), has shown efficacy in patients

treated in a 3 - 6 h window after stroke (Marks et al., 2008). Rodent stroke models provide the experimental knowledge for the determination of a relationship between extent of a lesion and resulting behavioral outcomes. In vivo stroke models are associated with relatively heterogeneous lesions in terms of size and location which, in turn, influences severity of behavioral impairment. Moreover, motor deficits are often modest when measured with gross motor assays such as rotarod, open field, or subjective scoring systems. We have previously reported that the use of a fine kinematic motor analysis to assess animal models of motor impairment highlights motor deficits that are not often evident with gross motor endpoints.
In this study, we used the transient middle cerebral artery occlusion (tMCAO) model to evaluate how duration of the occlusion affects size and location of lesion as well as the severity of neurological and motor deficits. Male Balb/c mice underwent tMCAO with 45 or 60 min occlusion time. Success of the occlusion was confirmed by diffusion-weighted MRI (DWI) directly before the reperfusion and structural T2-weighted MRI was performed 24 h later to assess lesion volume, edama and tissue viability. Neurological functions and motor deficits were assessed before the occlusion and then on day 1, 3,and 6 post-occlusion using neurological index and Composite Neuroscore, respectively. The mice were also subjected to fine motor kinematic gait analysis on day 3 and day 6 post-occlusion.
Analysis of lesion volumes between groups showed significant differences in volume size in the 60-min occlusion group with relatively low variability. The differences in lesion volume and edema were consistent with the degree of neurological and motor deficits in tMCAO mice compared to Sham operated animals on days 1. Similarly, the severity of motor deficits assessed with kinematic gate analysis on day 3 and day 6 significantly differed between sham and both occlusion groups and a trend towards higher severity in the 60-min group was present.
These data support the notion that stroke severity influence neurological and motor performance of animals. The tMCAO model in Balb/c mice provides homogeneous location and extent of lesions as well as produces significant behavioral and motor deficits as compared to healthy mice.

Disclosures: **M. Dudek:** None. **T. Bragge:** None. **A. Shatillo:** None. **A.J. Nurmi:** None. **D. Miszczuk:** None.

Poster

216. Stroke and Ischemia II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 216.25/G27

Topic: C.08. Ischemia

Title: Reorganization of the neuronal nucleoli in the neocortex, hippocampus and amygdala of white rats after acute ischemia

Authors: *V. AKULININ, A. STEPANOV, D. AVDEEV, A. GORBUNOVA;
Omsk State Med. Univ., Omsk, Russian Federation

Abstract: Nucleolus (*Nucl*) is the key structure that is responsible for the coordination of the cellular response to a stress. It has been proven that it's size, morphology and localization in the nucleus can greatly vary under stress. In this study we compared structural features of the *Nucl* in the neocortex, hippocampus and amygdala of the white rats brain one, three, seven, 14, 21, and 30 days after 20-min bilateral occlusion of the common carotid arteries (OCCA). We used Nissl and Hematoxylin-Eosin staining, DNA staining (DAPI), electron microscopy, and morphometry to analyze the changes. All experiments were performed in a strict compliance with the principles of humane treatment of animals. Morphometric analysis of images was performed using ImageJ 1.46. Animals in the control group for the most part had normochromic neurons with round smooth *Nucl* and an insignificant amount of condensed chromatin on the periphery of the cells. *Nucl* of almost all normochromic neurons belonged to the reticular structural type and bore signs of a high transcriptional activity. Animals subjected to OCCA demonstrated heterogeneity of morphofunctional activity of *Nucl* even among the normochromic neurons, perhaps as a result of a decline in the adaptive potential of some cells. We also observed a statistically (p <0.05) increase in the proportion of normochromic neurons with two or more *Nucl* (neocortex − 21.5%, CA1 hippocampus − 34.0%, CA3 − 17.4%, amygdala − 15.1%), despite the general trend towards the irreversible destruction and elimination of neurons. The highest amount of such two nucleoli neurons has been observed three days after OCCA (neocortex − 31.5 (95% Cl: 25.1-38.2), CA1 hippocampus − 22.8 (95% Cl: 17.2-29.3), and CA3 hippocampus − 43.6 (95% Cl: 36.6-50.8). No statistically significant differences were found for the amygdala. Normally, this figure was, respectively: 7.6 (95% Cl: 4.3-12.2), 8.5 (95% Cl: 5.0-13.3) and 15.0 (95% Cl: 10.4-20.7). The indicator dropped to the control levels 14 days after the OCCA. Nuclei in the 15-20% of neurons also had large clusters of inactive condensed chromatin, and decreased content of the RNP particles. This leads to a fall in the synthesis of information RNA, even with the activation of two or more nucleolar organizers. It was particularly true for the neocortex and CA1 hippocampus. The adaptive and reparative changes of *Nucl* did not provide the proper level of activation of the neuron's protein synthetic machinery. On the other side, functioning neurons had properly activated adaptation processes which were manifested by hypertrophy and amplification of the *Nucl*. This was particularly evident in the CA3 hippocampus.

Disclosures: V. Akulinin: None. **A. Stepanov:** None. **D. Avdeev:** None. **A. Gorbunova:** None.

Poster

216. Stroke and Ischemia II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 216.26/G28

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: Agencia Nacionalde Promoción Científica y Tecnológica PICT 1563, PICT 2731
University of Buenos Aires (20020130100564)
Consejo Nacional deInvestigaciones Científicas y T écnicas (PIP 0707)

Title: Critical role of monocyte recruitment in optic nerve damage induced by experimental optic neuritis

Authors: *M. ARANDA[1], D. GUERRIERI[2], G. M. PIÑERO[3], M. GONZÁLEZ FLEITAS[4], D. DORFMAN[5], R. E. ROSENSTEIN[6];
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Abstract: Neuroinflammatory diseases are characterized by blood-brain barrier disruption (BBB) and leukocyte infiltration. We investigated the involvement of monocyte recruitment in visual pathway damage provoked by primary optic neuritis (ON) induced by a microinjection of bacterial lipopolysaccharide (LPS) into the optic nerve from male Wistar rats. Increased Evans blue extrava-sation and cellularity were observed at 6 h post-LPS injection. In WT-GFPþ/WTchimeric rat optic nerves, the presence of GFP(+) neutrophils and GFP(+) monocytes, and in wild-type rat optic nerves, an increase in CD11b+CD45lowand CD11b+CD45high cell number, were observed at 24 h post-LPS. Gamma-irradiation did not affect the increase in BBB permeability, but significantly lessened the decrease in pupil light reflex (PLR), and retinal ganglion cell (RGC) number induced by LPS. At 6 h post-LPS, an increase in chemokine (C-C motif) ligand 2 (CCL2) immunoreactivity co-localized with neutrophils (but not microglia/macrophages or astrocytes) was observed, while at 24 h post-injection, an increase in Iba-1-immunoreactivity and its co-localization with CCL2 became evident. The co-injection of LPS with bindarit (a CCL2 synthesis inhibitor) lessened the effect of LPS on PLR, and RGC loss. The treatment with etoposide or gadolinium chloride that significantly decreased peripheral monocyte (but not neutrophil or lymphocyte) percentage decreased the effect of LPS on PLR, and RGC number. Moreover, a negative correlation between PRL and monocyte (but not lymphocyte or neutrophil) percentage was observed at 7 days post-LPS. Taken together, these results support that monocytes are key players in the initial events that take place during primary ON.

Disclosures: M. Aranda: None. **D. Guerrieri:** None. **G.M. Piñero:** None. **M. González Fleitas:** None. **D. Dorfman:** None. **R.E. Rosenstein:** None.

Poster

216. Stroke and Ischemia II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 216.27/G29

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH Grant R01NS082283

Title: Cln6-BioID identifies protein cargoes within ER-associated vesicles

Authors: *C. D. BOOTH[1], J. T. CAIN[1], T. B. JOHNSON[2], K. WHITE[1], C. SWANSON[1], J. BRUDVIG[1], R. N. LAUFMANN[4], D. MAY[1], K. J. ROUX[1], J. M. WEIMER[3];
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Abstract: CLN6 is a transmembrane endoplasmic reticulum (ER) associated protein that, when mutated on both chromosomes, causes the fatal neurodegenerative CLN6-Batten disease. We show that the CLN6 protein localizes to the ER as well as vesicles positive for ER markers suggesting that CLN6 may regulate the transport of specific vesicular cargo payloads. This study aims to identify proteins that interact with CLN6 and the cargo enriched within these CLN6-positive vesicles. Identifying CLN6 interactors and the cargo carried in these CLN6-postive vesicles is critical for understanding the nature of CLN6-Batten disease and for developing targeted therapies. Here we developed a BioID paradigm that labels proteins that interact with CLN6 by attaching a promiscuous mutant biotin ligase (BirA) to CLN6 protein at the luminal N-terminus. With proximity dependent biotinylation via the BirA enzyme, this construct biotinylates proteins that have proximal interactions with the CLN6 protein. These interacting proteins were streptavidin-affinity captured, purified, and identified using mass spectrometry. The BioID approach identified 35 unique candidate CLN6 interactors with diverse cellular roles, including a group of proteins that have been implicated in glial function and neurodegeneration as well as a group of proteins involved in cellular iron homeostasis and oxidative stress. New phenotypes were found based on our exploration of microglia function and splenic iron accumulation in the absence of CLN6.

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Poster

217. Spinal Cord Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 217.01/G30

Topic: C.11. Spinal Cord Injury and Plasticity

Support: National Institutes of Health/National Institute of Neurological Disorders and Stroke
US Department of Veterans Affairs

Title: Corticospinal and reticulospinal contribution to flexor and extensor arm muscles following spinal cord injury

Authors: *S. SANGARI[1,2], M. A. PEREZ[1,2,3];
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Abstract: The corticospinal and reticulospinal tract are major descending motor pathways contributing to the generation of movement. The corticospinal pathway largely facilitates contralateral flexor and extensor muscles while evidence showed that stimulation of the reticular formation facilitates ipsilateral flexor and contralateral extensor muscles of the forelimb. The goal of our study was to examine the contribution of the corticospinal and reticulospinal pathway to the control of elbow flexor and extensor muscle in humans with and without spinal cord injury (SCI). The contribution of the corticospinal pathway was examined by measuring the maximal voluntary contraction (MVC) and the input-output motor evoked potential (MEP) recruitment curves in biceps and triceps brachii muscles, elicited by transcranial magnetic stimulation over the arm motor cortex. The contribution of the reticulospinal pathway was explored by using the StartReact response by measuring reaction time from biceps and triceps brachii muscles electromyographic activity during isometric flexion and extension of the arm, respectively, in the presence of a startle acoustic stimuli. We found that SCI participants exhibited similar MVC and MEP-max but shorter reaction time during a startle stimuli in biceps brachii muscle compared with controls. However, SCI participants had reduced MVC and MEP-max and shorter reaction time during a startle stimuli in triceps brachii muscle compared with controls. Our results indicate that reticulospinal control of flexor and extensor muscles of the arm is enhanced after incomplete SCI and may play a role in motor function recovery following altered corticospinal control.

Disclosures: S. Sangari: None. **M.A. Perez:** None.

Poster

217. Spinal Cord Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 217.02/G31

Topic: C.11. Spinal Cord Injury and Plasticity

Support: VA BLR&D 1I01BX004039-01A2
Advancing a Healthier Wisconsin Endowment

Title: Absence of IL-12p40 mediates a beneficial effect on recovery after spinal cord injury

Authors: *J. ROSAS[1,2], N. PELISCH[1,2], B. APERI[1,2], K. STEHLIK[1,2], K. SWARTZ[1,2], A. KRONER-MILSCH[1,2];
[1]Neurosurg., Med. Col. of Wisconsin, Wauwatosa, WI; [2]Clement J. Zablocki Veterans Affairs Med. Ctr., Milwaukee, WI

Abstract: Traumatic spinal cord injury (SCI) is a relatively frequent event that imposes a massive burden on the health, quality of life and socioeconomic situation of affected persons and their caregivers. Both localization and extent of tissue damage in the injured cord influence the functional outcome. Tissue damage after SCI occurs in two phases. While primary damage describes tissue loss caused by the initial trauma, the lesion is expanded by secondary damage processes, including inflammation, hemorrhage, edema and production of reactive oxygen species. A more complete understanding of individual contributors to inflammatory damage is necessary to specifically target and modify detrimental factors. Inflammation after SCI is exacerbated, with activated microglia and monocyte-derived macrophages being the main immune cell populations in the injured tissue. Of particular interest are the pro-inflammatory cytokines IL-12 and IL-23, which share a subunit (p40) that is strongly upregulated after phagocytosis of red blood cells. IL-12 and IL-23 are expressed by a variety of cell types and have critical functions in regulating both the adaptive and innate immune system by inducing the production of pro-inflammatory cytokines. We are assessing the role of IL-12 and IL-23 and their receptors after SCI, using a low thoracic (T11) moderate contusion injury (50 kDyne) in mice. First, we quantified protein expression using western blot. IL-12p40 is significantly upregulated 24h after injury and stays upregulated throughout the observation period (28 days). The receptor subunit IL-12Rβ2 was also significantly upregulated, while IL-12Rβ1 and IL-23p19, the second subunit of IL-23, were not significantly changed. We utilized IL-12p40 and IL-23p19 knockout mice and wildtype controls to quantify locomotor recovery after SCI using the Basso Mouse Scale (BMS). We observed improved recovery in IL-12p40 deficient mice compared to wildtype mice. IL-12p40 KO mice demonstrated significantly more spared tissue and less iron accumulation at 28 days after SCI. In contrast, IL-23p19 KO mice did not differ from wildtype mice regarding their locomotor scores. In addition, we sought to identify the effect

of locally applied rIL-12 on locomotor recovery and inflammation after SCI. Our preliminary data suggest that local application of rIL-12 does not change locomotor recovery compared to vehicle treated controls. In summary, these results suggest that the absence of IL-12p40, but not IL-23p19, mediates a neuroprotective effect after contusion SCI, serving as a new potential therapeutic target to ameliorate secondary damage and promote better functional recovery outcomes.

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Poster

217. Spinal Cord Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 217.03/G32

Topic: C.11. Spinal Cord Injury and Plasticity

Support: GACR 17-03765S
CZ.02.1.01/0.0./0.0/15_003/0000419

Title: Effect of conditioned media of mesenchymal stem cells in rat model of spinal cord injury

Authors: *M. CHUDICKOVA, I. VACKOVA, L. MACHOVA URDZIKOVA, S. KUBINOVA;
Inst. of Exptl. Med., Prague, Czech Republic

Abstract: Application of conditioned media (CM) derived of mesenchymal stem cells (MSCs) is an admitted approach how to bypass limitations of direct MSC transplantation while preserving the beneficial effect of MSC on wounded tissues (anti-inflammatory, anti-apoptotic and trophic effect). Here we compared the effect of application of MSCs and their CM in the treatment of spinal cord injury (SCI) in Wistar male rats. We used MSCs derived from Wharton's jelly of umbilical cord (WJ-MSCs), which display high proliferative potential and produce large amounts of neurotrophic and growth factors. 1. 5M of MSCs and their CM, pooled from three different donors, were intrathecally transplanted in three doses 1st, 2nd and 3rd week after the induction of balloon compression lesion and animals were tested for 7 following weeks. The behavioural tests (Beam walk, BBB-test, plantar test) showed significant improvement after all three treatments (CM, WJ-MSCs and non-conditioned concentrated medium based on insulin-transferrin-sodium selenite supplement), in comparison with saline-treated controls. Moreover, there were significant improvements in Beam walk time / score measurements and plantar test in CM group, when compared to WJ-MSCs group, e.g. 7 weeks after SCI the Beam walk score in CM group was significantly higher; 3.74±0.23, in comparison to 2.66±0.27 in WJ-MSCs group, $p \leq 0.001$. Cresyl-violet and Luxol-fast blue staining of cross-sectioned spinal cords revealed

significant improvements after all three treatments in both grey and white matter sparing, in comparison with saline-treated controls, especially cranially from the centre of the lesion. In this area, CM group tends to show highest improvement, yet without any significance. To evaluate the level of astrogliosis after SCI, immunohistochemical staining of GFAP was employed. Although there was no significant difference in a size of glial scar among all tested groups, numbers of reactive astrocytes were significantly lower in CM group, when compared to both WJ-MSC group and group of saline-treated controls. Again, this effect was more particular cranially from the centre of the lesion. We conclude that we found statistically significant improvement after CM application in comparison with WJ-MSCs in Beam walk time / score measurements and plantar test, as well as significantly lower number of reactive astrocytes after CM application in comparison with WJ-MSC group.

Disclosures: M. Chudickova: None. **I. Vackova:** None. **L. Machova Urdzikova:** None. **S. Kubinova:** None.

Poster

217. Spinal Cord Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 217.04/G33

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Quantification of intrinsic hand muscle atrophy after human cervical spinal cord injury using ultrasound imaging

Authors: *C. S. KLEIN, H. LIU, Z. J. MENG, C. N. ZHAO, X. H. YANG;
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Abstract: Traumatic cervical spinal cord injury (SCI) often leads to paralysis of the intrinsic hand muscles that reduces their strength and function. Presumably, atrophy of these muscles also occurs, contributing to impaired ability to grasp objects. Our purpose is two-fold: (1) To develop a standardized method to quantify intrinsic hand muscle morphology using ultrasound imaging, and (2) to quantify morphological properties of these muscles in SCI persons and matched uninjured controls. Ultrasound images were recorded from the right abductor pollicis brevis (APB), flexor pollicis brevis, opponens pollicis, and first dorsal interosseous, and analyzed using ImageJ software. To date, we have studied 6 persons with C4-C7 chronic SCI (37 ± 14 y, 1.73 ± 0.04 m, 56 ± 9 kg, 6-13 months post injury), 4 of whom were complete (ASIA A) and 2 incomplete (ASIA B, C). Three controls (32 ± 2 y, 1.70 ± 0.06 m, 64 ± 10 kg) were also tested. The SCI subjects were in-patients undergoing further rehabilitation. Four SCI subjects had no voluntary control of the thumb and index finger; manual muscle test (MMT) score = 0. MMT scores were 4/1 and 1/1 for the thumb/finger in 2 others. Thickness and cross-sectional area of the different muscles were smaller (-30% to -50%, on average) in 4 SCI subjects compared to the

controls. This amount of atrophy was lessened by about 7% in each muscle when individual differences in distal thumb and index finger length were accounted for (ie., APB thickness/thumb length ratio). Grey scale analysis of the APB indicated a 10% higher pixel intensity in the 4 subjects, possibly indicating a larger proportion of non-contractile tissue. In contrast to these 4 subjects, one subject had preserved muscle size, despite complete paralysis. Another (thumb MMT= 4, pinch force = 2 kg, index finger MMT=1) also had preserved (or even larger) muscle size, possibly related to his 20+ years as a construction worker prior to his injury. These preliminary data suggest a variable amount of atrophy in intrinsic hand muscles after SCI, with some showing severe muscle loss and others showing little to no loss. Further experiments are needed to determine whether the severity of atrophy is related to characteristics of the injury, rehabilitation, and work history.

Disclosures: C.S. Klein: None. **H. Liu:** None. **Z.J. Meng:** None. **C.N. Zhao:** None. **X.H. Yang:** None.

Poster

217. Spinal Cord Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 217.05/G34

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NREF Young Clinician Investigator Award
Cervical Spine Research Society: Seed starter grant

Title: Multimodal parcellated study of the human connectome in patients with advanced cervical myelopathy

Authors: *D. S. JAYASEKERA[1], M. F. GLASSER[3], T. FRANK[2], A. H. HAWASLI[4]; [1]Biomed. Engin., Washington Univ. in St Louis, St Louis, MO; [2]Neurosurg., Washington Univ. in St Louis, St. Louis, MO; [3]Dept. of Anat. & Neurobio., [4]Dept. of Neurosurg., Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: Introduction: Despite being the most common form of spinal cord injury (SCI), there are insufficient diagnostic tools to evaluate cervical myelopathy (CM). There are no reliable methods to measure changes in brain architecture or functional networks after CM.
Rationale and Objective: The literature shows cerebral structural and functional connectivity changes in CM. However, variability in subject cohorts and prior techniques has led to divergent findings. Our exploratory study aims to develop, validate and implement a Human Connectome Project analysis to generate connectomes for CM.
Methods: Imaging and preprocessing methods are based on the HCP pipelines. T1 and T2 scans were obtained for cortical thickness and myelin mapping in the right and left hemispheres (RH

and LH). For functional imaging, multi-band gradient echo EPI sequence responsive to BOLD was used while subjects focused on a cross. To address experimental heterogeneity and maximize precision, we used a multi-modal MRI HCP approach for precise multi-modal brain mapping, improved spatial fidelity and neuroanatomical localization. Advanced CM subjects were compared to age and sex-matched healthy controls with equivalent educational levels.
Results: Using the HCP methodology, we have been able to generate high resolution structural and functional MRI datasets for subjects and controls. Respecting gyral anatomy and folds using the HCP methods have allowed us to maximize precision within the cerebral cortices. Early analyses have demonstrated discrete changes in select cortical thickness and myelin content within select cortical parcels involved in motor execution or sensory processing. Ongoing analyses include Cohen's d analysis of each cortical parcel and sub-parcels relative to controls with unpaired t-test's and multi-comparison correction. We are also generating functional connectivity matrices within and between HCP parcels and subparcels.
Conclusions: This research will provide a framework to assess how CM affects cortical myelin content, cortical thickness and functional connectivity within cortical regions involved in motor control and sensory processing. This method and data will serve the basis for further studies to develop connectomes for other spinal disorders and biomarkers for clinical decision-making and identification of new targets for therapies.

Disclosures: D.S. Jayasekera: None. **M.F. Glasser:** None. **T. Frank:** None. **A.H. Hawasli:** None.

Poster

217. Spinal Cord Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 217.06/G35

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H Neilsen Foundation 546893

Title: Urinary function after acute, sub-acute, and chronic spinal cord injury

Authors: *J. H. GUMBEL[1], C. HUBSCHER[2];
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Abstract: Urinary function is commonly disrupted after spinal cord injury (SCI) as is evident by the presence of polyuria, urinary tract/bladder infections, detrusor-sphincter dyssynergia, incontinence, and urinary retention. Due to the commonality of these issues, bladder/urinary function is ranked as a top priority as a quality of life measure in need of improvement by the SCI community. Using a contusion adult male rat model for SCI, we investigated differences in

upper and lower urinary function measures at acute, sub-acute, and chronic time points. Analysis of metabolic cage void and drink volumes indicated a significant increase in urine output at sub-acute (7-, 14- and 84-days post-SCI) but not acute (3-days) time-points post-injury. Sham animals showed no significant changes in urine output and drink volumes were not significantly different from pre-injury to post-injury across all groups, demonstrating the increase in urine output is not due to an increase in water intake. At termination, cystometry (CMG) with external urinary sphincter (EUS) electromyography (EMG) was performed to assess the lower urinary tract. The inter-contraction interval, void efficiency, baseline pressure, average non-void contractions, and inter-burst duration were all significantly altered after SCI at 3, 7, 14, and 84 days ($p < 0.05$ across all measures). The maximum amplitude of contraction was significantly decreased after SCI in the 3, 7, and 84 day SCI animals compared to sham. The time of bladder contraction per void was only significantly increased at the acute (3-day) time-point compared to both sham and all other SCI groups. This data from male rats demonstrates upper urinary dysfunction causing polyuria beginning sub-acutely and lasting for the chronic study duration post-SCI as well as significant bladder dysfunction immediately post-SCI. Because many of these deficits in urinary function are immediate and do not spontaneously improve over time, there is a critical need for early rehabilitation and pharmacological interventions.

Disclosures: **J.H. Gumbel:** None. **C. Hubscher:** None.

Poster

217. Spinal Cord Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 217.07/G36

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH/NINDS 1R01NS109552

Title: Exploration of reciprocal inhibition during electrical stimulation of spinal cord micro circuits

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Abstract: Spinal cord stimulation is widely used in patients with chronic pain, and electrical activation of spinal circuits has vast potential as an assistive therapy for individuals with spinal injury (Formento et al., 2018; Harkema et al., 2011; Minassian et al., 2004; Roy, Harkema, & Edgerton, 2012). However, the neurophysiology of spinal stimulation is still relatively unknown. Here we explore the mechanisms of direct electrical stimulation (DES) of the dorsal surface of the spinal cord. We use DES to stimulate the dorsal surface of the lumbar enlargement and on the

behavior of the canonical motor micro circuit (CMM) of the ankle flexor and extensor pair (Sol/TA) in the decerebrate cat. CMMs are comprised of Ia sensory axons, interneurons (IN) and motoneurons (MN), are repeated segmentally throughout the spinal cord and form the basis of reciprocal inhibition between agonist/antagonist muscle pairs and their proper function is critical for inter-joint coordination. Stimulation of the Sol/Ta CMM (10Hz-40Hz, 500μA, 5s) generated distinct force patterns comprised of 3 phases: an initial transient spike at the start of the stimulus, a profound inhibition during the duration of the stimulus and a long (< 15 s) sustained phase (rebound) after removal of the stimulus in the Sol muscle, and one phase, strong activation during the stimulation period, in the TA muscle. The force responses of TA and Sol are essentially mirror opposites during stimulation due to their reciprocal inhibitory relationship. The rebound force in Sol could illustrate the activation of persistent inward currents (PICs) or the interaction among inter neurons, allowing strong muscle activation when the stimulation ceased (rebound). This response pattern can be modulated by electrode location, favoring activation of TA or Sol. During stimulation, TA force is favored at the rostral end of the enlargement while Sol force is favored on the caudal end. Furthermore, the amount of rebound force in Sol is greatest when stimulating the rostral aspect of the enlargement. Thus it appears that Sol rebound force is modulated by the inhibitory strength from TA. Finally, acute thoracic transection (T12) abolished rebound activity in the Sol muscle but did not alter the interplay between Sol and TA. Rebound could not be induced even at stimulation intensities 4 times the stimulation used on the intact spinal cord suggesting dependence on a supra spinal mechanism. These results suggest that the excitatory/inhibitory balance between motor pools can be effectively modulated by electrical stimulation of the CMM via dorsal spinal stimulation and that there exist a fundamental difference between intact and transected spinal cord.

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Poster

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Program #/Poster #: 217.08/G37

Topic: C.11. Spinal Cord Injury and Plasticity

Support: MOST107-2314-B-038-041-MY3
MOST107-2321-B-001-020
MOST106-2321-B-001-044
TMU107-AE1-B24

Title: Muscle pain in lumbar radiculopathy

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Abstract: Introduction: Misdiagnosis of symptomatic lumbar lateral stenosis (LS) results in an unfavorable prognosis after surgical treatment. In our previous study, standardized qualitative sensory test has been proved to be a good diagnostic tool for the detection of LS, however, it is not suitable for patients with previous cutaneous sensation disorders on affected dermatome, such as previous trauma and diabetes mellitus associated peripheral neuropathy. Myotome is an alternative, but L5 nerve root innervates a group of muscles. Muscle pressure pain threshold (MPPT) is a quantitative tool for evaluation of affected muscles. This purpose of this study was to determine the affected muscles in patients with symptomatic LS-related lumbar radiculopathy (LR) and animals LR model. **Materials and Methods:** Human study: Three groups were prospectively recruited: 1. patients with LS-related lumbar radiculopathy 2. patients with central stenosis (CS)-related lumbar radiculopathy, and 3. healthy subjects. To test the reliability of MPPT, the difference of MPTTs between bilateral radialis muscles was measured and the difference less than 25 kPa was regarded as a good reliability. MPPTs of tibialis anterior (TA), gastrocnemius muscle medial head (GEM) and lateral head (GEL) on bilateral lower limbs are measured by algometer. The differences of muscle PPTs between two legs in patients with lumbar radiculopathy were calculated by: The difference of muscle PTT = PPT on the unaffected side - PPT on the affected side. Animal study: Three animal LR models were conducted: 1. Nerve ligation distal to L4 dorsal root ganglion, 2. Sham, and 3. Naïve. The MPPTs of TA and GE were measured during one week before operation to 12th weeks after operation. **Results:** There were no significant differences in age and gender between patients with LS-related and CS-related L5 radiculopathy. Patients with LS-related L5 radiculopathy presented a significant decrease of MPPT only in GEM muscle compared to patients with CS-related L5 radiculopathy and the health subjects. Interestingly, the decrease of MPPT was not observed in TA or GEL muscles. Similarly, mice with distal nerve ligation showed a significant decrease of MPPT in GE muscle. Interestingly, compatible to the observation from the patients with LS-related LR, the decrease of MPPT existed only in GE muscle not in TA muscle. **Conclusions:** According to MPPT, GEM was the affected muscle both in patients with symptomatic LS-related L5 radiculopathy and animal LR models. In the future, the underlying mechanism should be explored in animal LR models, and the affected muscle in L4, S1 radiculopathy should be determined.

Disclosures: Y. Yu: None. **Y. Chen:** None. **C. Chen:** None. **J. Lin:** None.

Poster

217. Spinal Cord Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 217.09/G38

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Western Schulich School of Medicine & Dentistry

Title: Decompression surgery in a rat model of cervical spondylotic myelopathy improves sensory but not motor recovery

Authors: N. M. GEREMIA[1], K. RYAN[1], S. GONCALVES[1,2], R. BARTHA[1], N. DUGGAL[3], *A. BROWN[1];
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Abstract: Cervical spondylotic myelopathy (CSM) is a degenerative disease that causes compression of the spinal cord and consequent locomotor and sensory dysfunction. Decompression surgery can arrest neurological decline but recovery itself remains highly variable. We developed a rat model of CSM using a hydrogel with delayed swelling characteristics that expands slowly over a period of time mimicking the slow progression observed in chronic compressive myelopathy. After testing various thicknesses, a hydrogel of 0.6mm thickness was found to cause a progressive neurological decline but not immediate paralysis. A 3 mm x 2 mm x 0.6 mm piece of hydrogel was placed under the C4/C5 laminae of Wistar rats by removing the ligamentum flavum at those spinal levels. At 6 weeks post-compression, rats were randomized to undergo surgical decompression to remove the hydrogel or sham surgery. MRI was used to monitor the hydrogel expansion and cord compression. The hydrogel expanded about 2 fold in size within 12 days, reaching 2.4 fold increase by 26 days. MRI confirmed spinal stenosis which was reduced by decompression surgery. Solochrome staining confirmed changes to the spinal cord cross-sectional area and loss to the white and gray matter. Rats were tested pre and post decompression for locomotor (grip strength) and sensory (mechanical allodynia) function. Compression of the cord resulted in a decrease in grip strength and an increase sensitivity to mechanical stimuli starting at 1 week post-compression. Decompression surgery improved forelimb mechanical allodynia marginally and halted the progression of hindlimb mechanical allodynia but did not improve grip strength. Sprouting of the primary afferent arbour in the spinal cord will be evaluated by CGRP immunohistochemistry as a possible mechanism for the allodynia observed. At 24 hrs post-decompression, neutrophil infiltration measured by immunohistochemistry was significantly increased within the cord suggesting an ischemia-reperfusion injury. The ischemia-reperfusion injury triggered by surgical decompression may assist in neurological decline. Our model of CSM is a translationally relevant animal model which can allow the further investigation to test the efficacy of treatments following decompression.

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Poster

217. Spinal Cord Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 217.10/G39

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Faperj
CAPES
CNPq

Title: Effects of different doses of mesenchymal stem cells on functional recovery after compressive spinal-cord injury in mice

Authors: B. S. RAMALHO, **F. M. ALMEIDA**, F. M. PESTANA, C. A. PRINS, F. S. CARDOSO, D. R. CAVALCANTE, B. GUTFILEN, S. A. SOUZA, *A. M. B. MARTINEZ; UFRJ, Rio de Janeiro, Brazil

Abstract: Spinal cord injury (SCI) causes sensory and motor deficits that impair functional performance, and significantly impacts expectancy and quality of life. Despite advances in technology and rehabilitation, no effective therapies are available for patients with SCI, which remains a major medical challenge. Cell therapy, using several lineages of stem cells, has been considered a treatment with potential for functional repair after spinal cord injury. This study compared the efficacy of 3 different doses of mesenchymal stem cells (MSCs) administered by intraperitoneal injection (i.p.) as a therapeutic strategy for compressive SCI. For this purpose, we used adult female C57BL/6 mice that underwent laminectomy at T9 level, followed by spinal cord compression for 1 minute with a 30g vascular clip. The animals received an i.p. injection of MSCs ($8x10^4$, $8x10^5$ or $8x10^6$ in 500 μL) or DMEM (500 μL), one week after SCI. After transplantation, up to 8 weeks, we performed behavior testing using Basso Mouse Scale-BMS, global mobility test, ladder rung walking test, Von Frey digital test and pin prick test. After that, the animals were anesthetized and an electromyography was performed; than the animals were sacrificed and the samples were processed for light and fluorescence microscopy. *In vivo* scintilography revealed that MSCs migrated to several organs 2 hours after transplantation, and the quantitative biodistribution analysis confirmed this data, 24 hours after transplantation. Analysis of the samples revealed that the cells of the three MSC doses, administered i.p., were able to migrate to the injury site. The semithin sections analysis revealed several preserved fibers in the MSC groups. The number of myelin fibers was higher in the MSC $8x10^5$ group than in the MSC $8x10^4$ group. Furthermore, the results of cell transplanted groups revealed a better white matter preservation in comparison to the DMEM; and the white matter was also better preserved in the MSC $8x10^5$ group than in the MSC $8x10^4$ group; in addition, treated groups presented higher quantities of BDNF, NT-3 and NT-4 as compared to DMEM group. The animals that

received $8x10^5$ MSC had a significantly higher score on the BMS scale and a greater number of total steps in the ladder rung walking test than the animals in all other experimental groups, at the end of 9 weeks. In all other functional evaluations, the groups receiving the MSCs transplant demonstrated significant improvements compared to the DMEM group. Cell transplantation at density $8x10^5$ showed the best therapeutic potential, leading to significant tissue and functional improvements compared to the other two doses.

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Poster

217. Spinal Cord Injury I

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Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH NS088475
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US Department of Veterans Affairs I01RX002787
Wings for Life
Craig H. Neilsen Foundation

Title: Topological network analysis of multicenter high-frequency intra-operative measurements in patients with spinal cord injury suggests the optimal MAP range associated with positive motor outcomes

Authors: *A. TORRES-ESPÍN[1], J. HAEFELI[1], R. EHSANIAN[2], D. TORRES[1], C. ALMEIDA[1], J. R. HUIE[1], B. DIRLIKOV[2], D. MOROZOV[3], N. SANDERSON[3], C. G. SUEN[1], J. L. NEILSON[4], J. TALBOTT[1], G. T. MANLEY[1], S. S. DHALL[1], J. BRESNAHAN[1], M. BEATTIE[1], W. D. WHETSTONE[1], S. L. MCKENNA[2], J. Z. PAN[1], A. R. FERGUSON[1], ,. AND THE TRACK-SCI INVESTIGATORS[1];
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Abstract: Spinal cord injury (SCI) causes motor, sensory and autonomic dysfunction in various degrees, depending on the injury severity and location. The heterogeneity of SCI results in data complexity that can benefit from modern analytical approaches designed to deal with this complexity. For instance, using data-driven topological data analysis we have previously shown

that high mean arterial pressure (MAP) during surgery (ultra-acute phase) predicts long-term functional recovery of SCI rodent models (Neilson et al., 2015). Moreover, it has been shown that MAP values may predict poor outcomes for SCI patient in the ICU (Cohn et al., 2010; Hawryluk et al., 2015). These cross-species findings motivated the present multicenter study, applying a data-driven workflow reviewing 133 surgical records from patients with SCI in the ultra-acute phase. Retrospective intra-operative monitoring records (MAP, heart rate) and neurological outcome data were extracted from operating room high-frequency monitoring systems, nursing notes, electronic medical records and paper charts. Data were curated, cleaned and harmonized using a combined human-machine workflow involving a collaborative team of data scientists and clinicians. Using machine-learning analytics we extracted a similarity network graph of 118 patients from a low-dimensional space embedded using Isomap, a non-linear algorithm. This approach ensures robust topological extraction using metrics of 'persistent homology', indicating high network stability. Exploratory network analysis suggested average MAP and time outside of an optimum MAP range (hypotension or hypertension) during surgery was associated with lower odds of recovering at least 1 AIS motor grade. Logistic and LASSO regression confirmed these findings, revealing an optimal MAP range associated with maximal recovery. The results of this study extend conclusion form animals models demonstrating that deviation from an optimal MAP range during surgery for patients with SCI may predict poor neurological recovery and suggest new targets for intervention.

Nielson et al., Topological data analysis for discovery in preclinical spinal cord injury and traumatic brain injury.Nat Commun. 2015
Cohn et al., Impact of mean arterial blood pressure during the first seven days post spinal cord injury. Top Spinal Cord Inj Rehabil. 2010
Hawryluk et al., Mean Arterial Blood Pressure Correlates with Neurological Recovery after Human Spinal Cord Injury: Analysis of High Frequency Physiologic Data. J Neurotrauma 2015

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Poster

217. Spinal Cord Injury I

Location: Hall A

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Program #/Poster #: 217.12/G41

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Shriners Hospitals Grant 85800
NIH Grant R01NS109064

Title: Properties of amniotic fluid and their role in spinal cord injury in myelomeningocele

Authors: *B. KRYNSKA[1], J. ZIEBA[1], O. GORDIIENKO[1], K. JANIK[1], J. A. GERSTENHABER[2], G. M. SMITH[1];
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Abstract: Myelomeningocele (MMC) is the most severe and common form of Spina Bifida, which results in significant and life-long neurological disabilities, impaired quality of life, and difficult medical management. In MMC, the spinal cord is exposed to the amniotic fluid (AF) through openings in the overlying vertebrae and skin resulting in progressive spinal cord damage and astrogliosis at the MMC lesion site. Although MMC studies indicate the involvement of a hostile intrauterine environment in the prenatal damage to the exposed spinal cord, the cause of spinal cord damage has not been clearly defined. Hyaluronic acid (HA) is an important component of the AF, which surrounds and protects the fetus during growth and plays a significant role in the unique fetal wound healing process and tissue regeneration. The main objective of this study was to determine whether the AF levels of HA in fetal rats with MMC are different from those in age-matched normal controls and whether these differences could have an impact on the viscosity of AF. MMC was established using the retinoic acid-induced rat model, which is analogous to human MMC and develops the entire spectrum of disease severity. The HA levels of AF were examined using a specific enzyme-linked immunosorbent assay and the viscosity of AF was determined using a viscometer. In addition, the progression of spinal cord injury in MMC fetuses was assessed by immunohistochemical examination of astrogliosis. The results showed that there were no significant differences in the AF levels of HA between the MMC and normal fetuses at earlier stages of gestation. Compared with normal fetuses, the AF level of HA and the viscosity of AF were significantly lower in late gestational age MMC fetuses. Furthermore, these alterations in the AF properties in fetal rats with MMC appeared to coincide with the increased severity of spinal cord damage and astrogliosis at the MMC lesion site. In summary, these findings suggest that the reduction in the AF viscosity due to the low level of HA may exacerbate the effects of mechanical trauma on spinal cord damage at the MMC lesion site and open future investigations to deepen our understanding of mechanisms underlying MMC pathogenesis and ultimately creating novel therapies for MMC repair.

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Poster

217. Spinal Cord Injury I

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Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant 2R25GM060507
NIH Grant P20MD006988

Title: Effects of FABP4 inhibition on functional and autonomic recovery in rats following spinal cord injury

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Abstract: Fatty Acid Binding Protein 4 (FABP4) is a modulator of inflammation that promotes a pro-inflammatory environment not conducive to cell survival. Upregulation of this protein in cancer and chronic inflammatory diseases such as diabetes and metabolic syndrome is becoming increasingly important in the etiology of these conditions. Nevertheless, its presence and function in the context of spinal cord injury (SCI) has not been reported. The pathology of traumatic spinal cord injury (SCI) results from an initial mechanical insult followed by secondary, inflammatory, processes. The secondary phase of injury is characterized by a marked deregulation in lipid metabolism leading to an inflammatory response known to cause axonal dieback, neuronal and oligodendrocytic death, and expansion of the injury. Lipid binding proteins, like fatty acid binding protein 4 (FABP4), are known to guide macrophage differentiation, particularly in the presence of pro-inflammatory lipids. The present study (1) examines the spatial- temporal expression and functional context of FABP4 following SCI in injured spinal cord epicenters and (2) studies the effects of FABP4 inhibition on locomotor and bladder recovery in rats. We are the first to report the significant upregulation of FABP4 protein and mRNA and the effects of its inhibition on autonomic and functional recovery in the injured rat spinal cord. Our data indicates that intrathecal administration of the FABP4 inhibitor BMS309403 promotes autonomic bladder recovery and significantly improves locomotor function in treated rats when compared to vehicle controls. Furthermore, we found that inhibition of FABP4 promotes axonal regeneration in treated rats as demonstrated by GAP43 staining. We propose that FABP4 is an attractive candidate for modulating inflammatory responses in the injured spinal cord.

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Poster

217. Spinal Cord Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 217.14/G43

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Sexually dimorphic regeneration and cardio-metabolic responses after spinal cord injury

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Abstract: Spinal cord injury (SCI) has devastating consequences that often leaves patients with permanent disability and pain syndromes. While most studies use male animals to model SCI due to the increased prevalence of SCI in human males, the incidence of human females with SCI is increasing. Sexual dimorphism is well established as an important factor of many conditions, including cardio-metabolic alterations, yet there has been little evaluation of sexual dimorphism after SCI. Our study was designed to identify sexually dimorphic responses in motor and sensory recovery, cardiac function and fibrosis, body composition, and glucose metabolism in Sprague Dawley (SD) rats after SCI. SCI was induced on female (n=9) and male (n=10) rats via thoracic contusion and compared to sham surgery (n=6 per sex). Results showed extensive differences in the post-SCI response between males and females across most of the measured parameters. Motor regeneration of female rats significantly exceeded that of males. Cardiac function, as measured using echocardiography, showed significant alterations in left ventricular internal diameters, left ventricular ejection fraction and left ventricular fractional shortening within a single sex and between males and females after SCI. Cardiac fibrosis increased significantly in males after SCI compared to females. Dual-energy X-ray absorptiometry (DEXA) scanning showed significant changes in body fat percentages after SCI within a single sex and between males and females. These data underscore the importance of consideration of sex in evaluating the effects, especially cardio-metabolic differences, of SCI. Such sex-based differences in movement recovery and cardio-metabolism could prove extremely valuable when it comes to possible prognosis and treatment plans for patients following SCI. More research will be needed to determine what other differences exist, and to what extent, to further improve patient treatment and success.

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Poster

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Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H. Neilsen Foundation
Wings for Life Foundation
International Spinal Research Trust
Rick Hansen Institute
International Neuroinformatics Coordinating Center (INCF)

UCSF-BASIC
University of Alberta

Title: A data commons infrastructure for private SCI data sharing

Authors: *J. HUIE[1], A. TORRES-ESPIN[1], C. A. ALMEIDA[3], D. SCHWERZ DE LUCENA[5], D. REINKENSMEYER[5], J. L. BIXBY[6], V. LEMMON[7], D. S. MAGNUSON[8], J. L. NIELSON[9], J. SCHWAB[10], C. TAYLOR-BURDS[11], W. TETZLAFF[12], E. S. ROSENZWEIG[13], M. H. TUSZYNSKI[14], K. FOUAD[15], M. S. BEATTIE[16], J. C. BRESNAHAN[2], M. E. MARTONE[17], J. S. GRETHE[18], A. R. FERGUSON[4];
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Abstract: The complex and heterogenous nature of spinal cord injury (SCI) has thus far limited translational bench-to-bedside results. The traditional publishing model, in which SCI complexity is summarized by sparse univariate measures using simplistic significance testing (t-tests, ANOVA, Pearson correlations) has contributed to the reproducibility crisis. The wide variety of data collected on a single SCI subject include injury parameters, biochemical, histological, and behavioral outcome measures, represent a ‘big data’ problem, which will require modern data science solutions. The SCI research field now has the opportunity to leverage the multidimensional nature of SCI to drive scientific insights. To fully seize this opportunity, managing and sharing the rich trove of SCI data is essential. To facilitate data sharing within and between labs, we have recently developed the Open Data Commons for Spinal Cord Injury (odc-sci.org). As of April 2019, the ODC-SCI hosts 49 labs, with 152 unique datasets, representing a broad cross-section of preclinical SCI data. The goal of this odc-sci.org is to make SCI data FAIR (findable, accessible, interoperable and reusable) and provide mechanisms for both data management and open data citation. However, SCI researchers may collect sensitive data that needs to remain private, including data sets designed to meet regulatory approval (e.g., FDA), sensitive intellectual property, non-human primate studies, etc. We have now addressed this need with the development of a Private Data Commons for SCI (PDC-SCI). This infrastructure allows verified users to securely upload and organize data in a scalable fashion, just as in the odc-sci.org, but in a repository that is not public-facing. This private infrastructure is an ideal data solution for multi-lab transdisciplinary studies that require well-organized, scalable data commons for rapid data sharing within a closed, distributed team that requires an extra level of security and privacy. We will present features of the PDC-SCI through the use-case of the VA Gordon Mansfield SCI Consortium.

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Poster

217. Spinal Cord Injury I

Location: Hall A

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Program #/Poster #: 217.16/H1

Topic: C.11. Spinal Cord Injury and Plasticity

Support: DoD SCIRP
CH Neilsen Foundation
Wings For Life

Title: Blood RNA biomarkers for SCI: Using RNAseq and advanced analytics for discovering diagnostic and prognostic clinical tools

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Abstract: Spinal cord injury (SCI) is one of the syndromes in medicine that despite decades of efforts still lacks an FDA-approved fluid biomarker for diagnosing the severity of the injury and furthermore offering a prognosis about the functional recovery of patients. The discovery of a fluid biomarker for SCI is of utmost importance as it 1) will allow clinicians to rapidly assess the severity of the injury and the patient's recovery prospects, 2) will serve as a method to stratify patients for clinical trials, and most importantly 3) could be used as a biosensor for monitoring the efficacy of treatments. Many attempts have been made to identify fluid biomarkers but to our best knowledge almost all of them have been at the protein level and although they have given great insights to the field have not yet been universally established as SCI biomarkers. We used RNAseq for detecting the RNA levels of all genes in the peripheral white blood cells (WBCs) of SCI patients admitted at the Zuckerberg San Franscisco General Hospital and Trauma Center at multiple time points after the injury. Then we applied advanced analytics to correlate the gene

expression levels with the initial severity of the injury (measured by ISNCSCI/AIS grade) and the long-term functional recovery at 6 and 12 months post injury. Peripheral blood was collected within 24, 48, 72 and 120 hours post SCI. Samples from Healthy Controls (HCs) and non-CNS Trauma Controls (TCs) were also included in our study. After preprocessing of the RNAseq data, we used Weighted Gene Co-expression Network Analysis (WGCNA) and Principal Component Analysis (PCA) to correlate gene and gene module expression changes with a large dataset of clinical records we are collecting for our clinical study (TRACK-SCI). Most importantly, we are seeking correlations between genes/gene modules and ISNCSCI/AIS scores at the time of the injury and at 6 and 12 months post SCI. We sequenced samples from 38 SCI patients, 10 HCs and 10 TCs. The analysis displayed a clear transcriptomic separation between the three populations as shown by a multidimensional scaling plot. In addition, 2,423 genes were found to be differentially regulated upon SCI and 115 of them showed a directional correlation with the initial severity of the injury. In addition we identified genes correlated with known MRI markers as well as with the Mean Arterial Pressure (MAP) of the patients as measured at the time of transportation to the hospital. Lastly, our WGCNA approach identified novel gene modules whose expression pattern correlate very significantly with the SCI severity and show great potential to be used as diagnostic and prognostic tools.

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Poster

217. Spinal Cord Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 217.17/H2

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Ohio State University Center for Muscle Health and Neuromuscular Disorders
Pre-Doctoral Fellowship

Title: Neuromuscular connectivity and muscle function during acute spinal cord injury

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Abstract: Objective electrophysiological measures are needed for more effective cross-species translation, as prior work has uncovered pronounced fundamental differences in the anatomical and functional characteristics of the motor systems in primates versus rodents (Courtine et al.

2007; Friedli et al. 2015). The ability to factor in neurobiological differences has been limited and constitutes an obstacle for the translation of rodent models to patients. Spinal cord injury (SCI) research is one particular area where the ability to assess different spinal cord regions and pathologies (i.e. upper or lower motor neuron dysfunction) may be of particular benefit (Thomas et al. 2014). Additionally, low replicability and robustness of behavioral assays of functional recovery limit translation of findings in preclinical SCI models (Ahuja et al. 2017; Schaffran and Bradke 2019; Watzlawick et al. 2018). Therefore, objective measures of electrophysiological and physiological outcomes will optimize predictive value of experimental SCI studies. Whereas measures of upper motor neuron connectivity have been readily established in the rat, functional assessments of lower motor neuron innervation of forelimb muscles are lacking. Compound muscle action potential (CMAP) and motor unit (MU) number estimation (MUNE) are well-established methods that allow longitudinal monitoring of MU integrity in patients. In analogy we refined CMAP and MUNE methods for assessing spinal MU input in the rat forelimb and hindlimb. We longitudinally applied these electrophysiological techniques over a period of two weeks in rats with thoracic spinal cord injury. Muscle contractility was also measured and compared to electrophysiological measures. The forelimb and hindlimb recording techniques presented here can also be applied to experimental cervical SCI models, the most common level of SCI (Ahuja et al. 2017), to understand the combined effects of upper and lower motor neuron dysfunction. Moving forward, CMAP and MUNE measures are putative electrophysiological biomarkers of forelimb and hindlimb MU integrity poised to guide and improve bi-directional translation in experimental spinal cord and motor neuron disease models.

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Poster

217. Spinal Cord Injury I

Location: Hall A

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Program #/Poster #: 217.18/H3

Topic: C.11. Spinal Cord Injury and Plasticity

Support: China Scholarship Council
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Craig Neilsen Foundation Award 476975
Generous donations from Cure Medical, Research for Cure

Title: Selective activation of the cells of origin of the corticospinal tract using a 2-vector system for selective expression of DREADDs

Authors: ***J. LUO**[1,2], M. METCALFE[1], J. YONAN[1], K. MATSUDAIRA[1], A. GUNAWAN[1], J. MIZUFUKA[1], O. STEWARD[1];

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Abstract: Chemogenetics has emerged as a means of manipulating neuronal excitability, and potentially activity of selected populations of neurons. Here, we tested a two-vector system that enables selective activation of cells of origin of the corticospinal tract (cortical motoneurons) by designer drugs (DREADDs). Rosa^{tdTomato} mice, which express tdTomoto upon Cre expression, received intra-spinal injections of retro-AAV/Cre at cervical level 5, leading to retrograde transduction of neurons throughout the sensorimotor cortex. Mice also received unilateral intra-cortical injections of a Cre-dependent hM3Dq expressing AAV (AAV8-hSyn-DIO-HA-hM3D(Gq)-IRES-mCitrine). With this approach, only cortical motoneurons express Cre to mediate expression of DREADDs. Three weeks after intra-cortical viral injection, mice received clozapine-N-oxide (CNO) and were perfused 1.5hrs later, and brains were prepared for immunohistochemistry for tdT, HA, and mCitrine (assessed using a GFP antibody), and c-fos to assess activity-induced IEG expression. tdT expression marked cortical motoneurons throughout the sensorimotor cortex. HA and Citrine immunofluorescence revealed cortical motoneurons in which DREADDs expression had been induced. Immunostaining for c-fos revealed selective activation of cortical motoneurons in areas of HA expression. Citrine expression also allowed tracing of mCitrine-labeled corticospinal axons in the spinal cord. Taken together, these data document a feasible approach to selectively activate cortical motor neurons projecting to the spinal cord chemogenetically, definitively identify the activated neurons using immunocytochemistry, and selectively trace axons of the activated neurons in the spinal cord.

Disclosures: J. Luo: None. **O. Steward:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dr.Steward is a co-founder and holds economic interests in the company “Axonis”, which holds a license on patents relating to PTEN deletion and axon regeneration.. **M. Metcalfe:** None. **J. Yonan:** None. **K. Matsudaira:** None. **A. Gunawan:** None. **J. Mizufuka:** None.

Poster

217. Spinal Cord Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 217.19/H4

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig Neilsen Foundation Award 476975
Generous donations from Cure Medical, Research for Cure

Title: Harnessing retro-AAV for gene manipulations on multiple pathways that are interrupted by spinal cord injury

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Abstract: Spinal cord injury (SCI) interrupts the long tracts that interconnect the brain and spinal cord resulting in paralysis, loss of sensation and impairment of bladder, bowel, and sexual functions. Restoring multiple functions will require regeneration of multiple pathways, but interventions to achieve this goal are lacking. Previous studies have documented that deletion of Phosphate and tensin homolog (PTEN) enables robust axon regeneration after SCI, but a major limitation is that interventions have involved AAV-mediated deletion of PTEN in limited numbers of neurons that give rise to particular pathways such as the corticospinal tract (CST). Here, we document a new approach that allows simultaneous transfection of large numbers of neurons of origin of multiple spinal pathways. The approach uses retrogradely transported adeno-associated virus (retro-AAVs); with small injections into the spinal cord, retro-AAVs are robustly transported to the cells of origin of multiple spinal pathways. Proof of concept studies were carried out using $PTEN^{f/f}$;$Rosa^{tdTomato}$ mice in which transfection with retro-AAV/Cre deletes PTEN and activates tdT expression in the same neurons. Injections of retro-AAV/Cre into the cervical spinal cord (C5) led to retrograde transfection and induction of tdT expression in the cells of origin of multiple spinal pathways including the majority of cortical neurons that give rise to the CST as well as neurons in the red nucleus, reticular formation, and Barringer's nucleus, which controls bladder function. Immunostaining for PTEN revealed deletion of PTEN in tdT-positive neurons. Next, we engineered a retro-AAV expressing shRNA against PTEN along with a GFP reporter (retro-AAV-shPTEN/GFP), which in theory could be used in any species. As a proof of concept, rats received injections (retro-AAV/shPTEN/GFP) at C5; immunostaining for GFP revealed robust retrograde transfection of the cells of origin of multiple spinal pathways including large numbers of cortical neurons as well as neurons of origin of multiple descending brainstem pathways. Immunostaining for PTEN revealed that the level of shPTEN expression was sufficient to delete PTEN in transfected neurons. Together, our results document that retro-AAV provides a platform with remarkable potential for AAV-based gene-modifying interventions to enable regeneration of pathways that are interrupted by SCI.

Disclosures: M. Metcalfe: None. **K. Yee:** None. **J. Mizufuka:** None. **R. Azevedo:** None. **S.P. Gandhi:** None. **O. Steward:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); OS is a co-founder and holds economic interests in the company "Axonis", which holds a license on patents relating to PTEN deletion and axon regeneration..

Poster

217. Spinal Cord Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 217.20/H5

Topic: C.11. Spinal Cord Injury and Plasticity

Support: RO1-NS092876
SHC-85400
SHC-85220
SHC-84293

Title: Combinatorial treatment protects identified neurons in brain from retrograde apoptosis after spinal cord injury in lamprey

Authors: *J. HU[1], G. ZHANG[1], W. RODEMER[2], L.-Q. JIN[3], M. E. SELZER[4];
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Abstract: Paralysis following spinal cord injury (SCI) is due to axon interruption and failure of regeneration. Both extrinsic and intrinsic factors contribute to the inability of axon regeneration and neuronal survival. Removal of chondroitin sulfate proteoglycans (CSPGs) with chondroitinase ABC (ChABC) enhances axon growth and functional recovery after SCI, but in mammalian models, due to the complexity of the system, it is not clear how this treatment affects the downstream signalling pathways of CSPG receptors *in vivo*. The brainstem of the lamprey has individually identified reticulospinal (RS) neurons, which have defined perikaryal survival and axon regeneration probabilities after SCI. We took advantages of these identified neurons and found that ChABC application protects these neurons from retrograde apoptosis by greatly increasing Akt activation *in vivo*. We have reported that RhoA knockdown by retrogradely delivered morpholino antisense oligonucleotides (MOs) *in vivo* can reduce retrograde neuronal apoptosis after SCI. Recently we also found that RhoA knockdown *in vivo* enhanced Akt activation. To elucidate the relationships between ChABC and RhoA in neuronal survival *in vivo*, we have investigated whether ChABC adds to the neuronal protective effects of RhoA knockdown *in vivo*. Four different treatment combinations were applied in lampreys for 2 or 4 weeks after SCI: control MO with enzyme buffer, control MO with ChABC, RhoA MO with enzyme buffer, and RhoA MO with ChABC. Consistent with our previous findings on the effects of ChABC application and RhoA knockdown, there was less caspase activation in the Ctr MO + ChABC group than in the Ctr MO + enzyme buffer group, and the RhoA MO + enzyme buffer group had fewer identified RS neurons undergoing apoptosis compared to the group with Ctr MO + enzyme buffer. More excitingly, RhoA MO + ChABC had the best protective effect on

identified RS neurons among the four treatment combinations. This additive effect of ChABC treatment and RhoA knockdown suggests that at least some of the neurotoxic effects of CSPGs are not mediated by RhoA.

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Poster

217. Spinal Cord Injury I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 217.21/H6

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant R01NS092876

Title: The role of PTPσ in lamprey axon regeneration and cell death after spinal cord injury

Authors: *W. RODEMER[1], K. ZHANG[1], I. SINITSA[2], J. HU[1], M. E. SELZER[1,3];
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Abstract: Traumatic spinal cord injury (SCI) results in persistent functional deficits due to the lack of axon regeneration within the mammalian CNS. After SCI, chondroitin sulfate proteoglycans (CSPGs) inhibit axon growth via putative interactions with the LAR-family protein tyrosine phosphatases, PTPσ and LAR, localized on the injured axon tips. Unlike mammals, the sea lamprey, *Petromyzon marinus*, robustly recovers locomotor behaviors following complete spinal cord transection (TX). This recovery is accompanied by heterogeneous regeneration of the lamprey's reticulospinal (RS) system, whose identifiable neurons can be categorized as "good" or "bad" regenerators, based on the likelihood of regeneration of their severed axons. Those neurons that fail to regenerate their axons undergo a delayed form of apoptotic cell death. Previously, this lab reported that lamprey PTPσ mRNA is expressed preferentially in "bad regenerator" RS neurons, in advance of SCI-induced caspase activation. Consequently, we hypothesized that PTPσ deletion would reduce retrograde cell death and promote axon regeneration. Using antisense morpholino oligomers, we reduced PTPσ expression after TX, and assessed the effects on axon regeneration, caspase activation, intracellular signaling, and behavioral recovery. Surprisingly, PTPσ knockdown antagonized recovery resulting in mild behavioral deficits and, most strikingly, increased supraspinal cell death (~16% at +10wk, $p<0.01$; ~35% at +20wk, $p<0.001$). No differences in axon regeneration were observed among surviving RS neurons between controls and PTPσ knockdowns at 10 weeks post-TX. FLICA assays revealed no increase in caspase activation after PTPσ knockdown,

suggesting that cell loss may occur via non-apoptotic mechanisms. Although the mechanism of cell death in these experiments is not known, alternate studies in mammals suggest PTPσ deletion can exacerbate inflammation. Ongoing studies will determine whether PTPσ knockdown with morpholinos increases the inflammatory response to SCI, possibly via incidental transfection of infiltrating immune cells. Additionally, the failure of PTPσ deletion to improve regeneration could be, in part, due to compensation by LAR, which is co-expressed in the lamprey RS neurons. Future studies will assess the effects of LAR knockdown, alone or in combination with PTPσ, on retrograde cell death and axon regeneration in lamprey RS neurons.

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

Poster

218. Somatosensation: Ion Channels

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 218.01/H7

Topic: D.02. Somatosensation

Title: The role of TRPM2 in thermosensation and thermoregulation

Authors: *M. HUANG[1], C. GAO[2], W. SHEN[2], W. YANG[1];
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Abstract: TRPM2 is a nonselective calcium permeable cation channel, which can be activated by intracellular ADPR and extracellular hydrogen peroxide. It is also a temperature sensitive TRPs, opening at above 35℃. The recently studies have revealed that TRPM2 participated in warmth sensation and core body temperature regulation(T_{core}) during fever by using TRPM2 knockout mice. However, the contribution of TRPM2 in certain cell types on thermosensation and thermoregulation are still unclear. In this study, we used conditional TRPM2 knockout mice to investigate the role of TRPM2 in thermosensation and thermoregulation. Firstly, we found that TRPM2 mainly expressed in medium and small diameter dorsal root ganglion(DRG) neuron. Conditional knockout of TRPM2 in DRG neuron shifted thermal preference towards warmer temperature. Secondly, we also detected TRPM2 highly expressing in median preoptic area(MPO) of hypothalamus. TRPM2 selectively deficiency in MPO didn't affect T_{core} during PGE_2 inducing fever, but slowed down T_{core} restoration after heat stimulus. In summary, our results indicated that TRPM2 in DRG contributed to warmth sensation and TRPM2 in MPO played the critical role in T_{core} restoration.

Disclosures: M. Huang: None. **C. Gao:** None. **W. Shen:** None. **W. Yang:** None.

Poster

218. Somatosensation: Ion Channels

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 218.02/H8

Topic: D.03. Somatosensation – Pain

Support: DIP Grant BI 1665/1-1ZI1172/12 to 1
ISF Grant 470/17
Hoffman Leadership program
NIH Grant 1R01DA38860

Title: Platelet-derived growth factor contributes to inflammatory pain by inhibiting Kv7/M channels in nociceptive neurons

Authors: *O. BARKAI[1], S. PUIG[2], S. LEV[1], B. TITLE[1], B. KATZ[1], L. ELI-BERCHOER[1], H. B. GUTSTEIN[2], A. BINSHTOK[1];
[1]The Hebrew Univ. of Jerusalem, Jerusalem, Israel; [2]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Endogenous inflammatory mediators contribute to the pathogenesis of pain by acting on nociceptors, specialized sensory neurons that detect noxious stimuli. We describe the platelet-derived growth factor (PDGF)-BB as a new inflammatory pain mediator. Using patch-clamp recordings we show that PDGF-BB applied in vitro causes repetitive action potential firing in dissociated nociceptor-like rat dorsal root ganglion (DRG) neurons and decreases their threshold for action potential generation. Injection of PDGF-BB into the paw produces nocifensive behavior in rats and leads to thermal and mechanical pain hypersensitivity. Biophysical analyses of these PDGF-BB effects show that PDGF receptor-induced inhibition of nociceptive M-current underlies the mechanism behind PDGF-BB-mediated nociceptive hyperexcitability. Importantly, in vivo sequestration of PDGF or PDGF receptor inhibition attenuates formalin-induced acute inflammatory pain. Finally, we demonstrate that imatinib, a clinically used PDGFR inhibitor, attenuates the PDGF-BB-mediated hyperexcitabilty in vitro and reduces inflammatory pain in vivo. Our discovery of a new pain-facilitating proinflammatory mediator enhances our understanding of inflammatory pain pathophysiology and may have important clinical implications for the treatment of pain.

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Poster

218. Somatosensation: Ion Channels

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 218.03/H9

Topic: D.02. Somatosensation

Support: CIHR MOP159906
NSERC CG04499
Chaire de recherche cerveau et douleur 0487

Title: Distinct expression patterns of acid - sensing ion channels in mouse primary sensory afferents

Authors: *M. PAPALAMPROPOULOU-TSIRIDOU, F. WANG, Y. DE KONINCK;
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Abstract: It is well established that Acid- Sensing Ion Channels (ASICs) have been implicated in normal functions of the central and peripheral nervous system, as well as in a number of pathological conditions. Four genes (ASIC1-4), encoding 6 different subunits (ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3, and ASIC4) through alternative splicing, have been identified in rodents. ASICs and more specifically ASIC1a and ASIC3 have been shown to be implicated in pain since both subunits are expressed in nociceptors in the Dorsal Root Ganglia (DRGs). On the other hand, ASIC2 was found in mechanosensitive structures in rodent skin and was involved in touch sensation. ASIC1, ASIC2 and ASIC3 expression was identified in adult mouse DRGs, however their detailed expression pattern has not been investigated yet. The aim of the present study is to examine thoroughly the expression pattern of different ASIC subunits in different neuronal populations. To overcome the obstacle of poor specificity of anti-ASICs antibodies, we used a very sensitive in situ hybridization technique (RNAscope) to identify the expression of the genes of interest. We combined this methodology with immunohistochemistry to reveal specific neuronal populations in DRGs. More specifically we targeted Isolectin IB4 (IB4), Calcitonin gene-related peptide (CGRP) and Neurofilament 200 (NF200) positive neurons. All experiments were performed on frozen sections of fixed DRGs obtained from 4 naïve adult male mice. Based on our results, ASIC1a and ASIC1b are not expressed in $IB4^+$ neurons, but in about 28% of $CGRP^+$ and 72% of $NF200^+$ neurons. ASIC2a is expressed in about 70% of $IB4^+$, 11% of $CGRP^+$ and half of $NF200^+$ neurons. ASIC2b is expressed in most DRG neurons, including almost all $IB4^+$ and $CGRP^+$ neurons, as well as 80% of $NF200^+$ neurons. ASIC3 is expressed in 10%, 65% and 82% of $IB4^+$, $CGRP^+$ and $NF200^+$ neurons, respectively. Overall, different ASIC subunits show a distinct expression pattern in DRG neurons. The only two subunits with similar expression patterns are ASIC1a and ASIC1b; they are present in most of $NF200^+$ neurons but are not in $IB4^+$ neurons. In contrast, ASIC2a and ASIC2b have different expression pattern. ASIC2a

is mostly expressed in $IB4^+$ neurons while ASIC2b is present in most DRG neurons. Finally, ASIC3 is expressed in most $NF200^+$ neurons and in the majority of $CGRP^+$ neurons. The distinct expression patterns of different ASICs in DRG neurons, indicates that they may be involved in different types of somatosensation.

Disclosures: M. Papalampropoulou-Tsiridou: None. **F. Wang:** None. **Y. De Koninck:** None.

Poster

218. Somatosensation: Ion Channels

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 218.04/H10

Topic: D.02. Somatosensation

Title: The G protein-biased agents PZM21 and TRV130 are partial agonists of μ-opioid receptor-mediated signaling to ion channels

Authors: *Y. YUDIN, T. ROHACS;
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Abstract: Opioids remain the most efficient medications against severe pain; they act on receptors that couple to heterotrimeric G-proteins in the Gαi/o family. Opioids exert many of their acute effects through modulating ion channels via Gβγ subunits. Many of their side effects are attributed to β-arrestin recruitment. Several biased agonists that do not recruit β-arrestins, but activate G-protein dependent pathways have been developed recently. While these compounds have been proposed to be full agonists of G-protein signaling in several high throughput pharmacological assays, their effects were not studied on ion channel targets.
Here we used patch clamp electrophysiology and Ca^{2+} imaging to test the effects of TRV130, PZM21 and herkinorin, three G-protein biased agonists of μ-opioid receptors (μOR), on ion channel targets of Gαi/o/Gβγ signaling: G-protein activated inwardly rectifying K^+ (Kir3.2), N-type voltage gated Ca^{2+}-channel (Cav2.2) and Transient Receptor Potential Melastatin 3 (TRPM3) which is a recently described ion channel target for $G_{\beta\gamma}$ subunits. We also studied G-protein dissociation using a fluorescence resonance energy transfer (FRET)-based assay.
All three biased agonists induced smaller activation of G protein-coupled inwardly rectifying potassium channels (GIRK2), and smaller inhibition of Transient Receptor Potential Melastatin (TRPM3) than the full μOR agonist DAMGO. Co-application of TRV130 or PZM21, but not herkinorin alleviated the effects of DAMGO on both channels. PZM21 and TRV130 also decreased the effect of morphine on GIRK2 channels. CaV2.2 was also inhibited less by PZM21 and TRV130 than by DAMGO. We also found that TRV130, PZM21 and herkinorin were less effective than DAMGO in inducing dissociation of the Gαi /Gβγ complex.
Conclusion and Implications: TRV130, PZM21 and potentially herkinorin are partial agonists of μOR.

Disclosures: **Y. Yudin:** None. **T. Rohacs:** None.

Poster

218. Somatosensation: Ion Channels

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 218.05/H11

Topic: D.02. Somatosensation

Support: National Research Foundation of Korea (2011-0018358)

Title: Activation mechanism of Tentonin 3/TMEM150C

Authors: *G. HONG[1], **S. PAK**[2], J. CHOI[2], U. OH[2];
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Abstract: Tentonin 3/TMEM150C (TTN3) has been recently discovered as a novel mechanosensitive (MS) channel. It is readily activated by mechanical stimuli but inactivates slowly in heterologous system. Upon genetic ablation of Ttn3, the proportion of neurons with slowly-adapting (SA) MS currents is significantly reduced in dorsal-root ganglion neurons. Thus, TTN3 appears to confer the SA MS currents in dorsal-root ganglion neurons. However, a question was raised that TTN3 might be a regulatory protein of Piezo1 because TTN3 was not activated in *Piezo1*-deficient HEK (HEK-P1KO) cells (Dubin, Neuron 2017). To address this issue, we hypothesized that mechanosensitivity of TTN3 depends on cytoskeleton integrity of the cell. TTN3-MS current was markedly decreased by treatment of the cytochalasin D, an actin assembly disruptor. Thus, TTN3 mechanosensitivity depends on cytoskeleton integrity. Because Piezo 1 and 2 are known to promote the formation of stress fibers or focal adhesion and integrin-dependent cell migration, respectively, thus Piezo1 ablation in HEK cells (HEK-P1KO) may lower the membrane stiffness. Indeed, when the actin assembly is strengthened after jasplakinolide-treatment, mechanical stimuli robustly evoked MS currents in *Ttn3*-transfected HEK-P1KO cells. This result clearly suggest that TTN3 is activated by mechanical stimuli even in the absence of Piezo1. Supporting this hypothesis, the expressions of focal adhesion proteins were reduced in HEK-P1KO cells. Furthermore, knockdown of these molecules also reduced the TTN3-dependent MS currents. Suction of blebs of HEK cells expressing Piezo1 induced MS currents. However, suction of blebs expressing TTN3 failed to induce MS currents. Thus, the activation mechanism of TTN3 appears different from that of Piezo1. We concluded that mechanosensitivity of TTN3 is dependent on cytoskeleton integrity but not on Piezo1. This finding supports the idea that TTN3 is the essential component of slowly inactivating mechanosensitive channel.

Disclosures: **G. Hong:** None. **S. Pak:** None. **J. Choi:** None. **U. Oh:** None.

Poster

218. Somatosensation: Ion Channels

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 218.06/H12

Topic: D.03. Somatosensation – Pain

Title: Involvement of acid sensing ion channel 1b in the development of chronic muscle pain

Authors: *C.-T. CHANG[1], S.-W. FONG[1,2], C.-H. LEE[1], S.-H. LIN[3,4], C.-C. CHEN[1,2];
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Abstract: Acid-sensing ion channel 1b (ASIC1b), an amiloride-sensitive proton-gated Na^+ channel, predominantly expressed in the peripheral nervous system. Although ASIC1b was firstly identified almost two decades ago, the role of ASIC1b and the exact neuronal subtypes expressing ASIC1b is still elusive. Here, we generated transgenic mice with $ASIC1b^{cre}$ co-expressed tdTomato reporter to probe the functions of ASIC1b in nociception. We first validated the $ASIC1b^{cre}$ line by using in situ hybridization in the dorsal root ganglion (DRG) neurons and found ~90% $ASIC1b^{cre}$-$tdTomato^+$ neurons expressed ASIC1b mRNA. In whole-cell patch clamp recording, we found most medium- to large-diameter ASIC1b+ DRG neurons exhibited amiloride-sensitive biphasic current in response to acid stimulation, while most small- to medium- diameter ASIC1b+ DRG neurons exhibited amiloride-insensitive sustained current. Specifically, acid-induced biphasic current can be partially attenuated by APETx2 and Mambalgin-1 but not PcTx1 suggesting ASIC1b forms a wide variety of functional heteromeric channels with other ASIC subtypes. In a mouse model of fibromyalgia induced by dual intramuscular acid (pH4.0) injections, wild-type mice developed long-lasting hyperalgesia for at least 4 weeks. In contrast, mice lacking ASIC1b were not able to develop hyperalgesia by a single intramuscular acid injection; a second intramuscular acid injection spaced 5 days apart induced hyperalgesia lasting only three days. In conclusion, ASIC1b contributes to acid-induced chronic muscle pain via forming heteromeric channels with other ASIC subtypes.

Disclosures: **C. Chang:** A. Employment/Salary (full or part-time):; Institute of Biomedical Sciences, Academia Sinica, Taipei, 115, Taiwan. **S. Fong:** None. **C. Lee:** None. **S. Lin:** None. **C. Chen:** None.

Poster

218. Somatosensation: Ion Channels

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 218.07/H13

Topic: D.02. Somatosensation

Title: Ion channels involved in pain pathways: An automated patch clamp approach

Authors: *A. R. OBERGRUSSBERGER[1], **E. DRAGICEVIC**[1], N. BECKER[1], M. RAPEDIUS[1], T. A. GOETZE[1], M. G. ROTORDAM[1], N. BRINKWIRTH[1], I. RINKE-WEIß[1], S. STOELZLE-FEIX[1], C. HAARMANN[1], S. FRIIS[1], T. STRASSMAIER[2], R. HAEDO[2], M. GEORGE[1], A. BRÜGGEMANN[1], N. FERTIG[1];
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Abstract: Chronic and neuropathic pain is a significant problem affecting millions of people worldwide each year. A variety of different ion channels play significant roles in pain transmission including TRP, P2X, NMDA, Na_V and, more recently, HCN1 and 2. As a family of cation channels with roles in many biological functions TRP channels have been targets for pain perception and thermosensation. Among ligand-gated ion channels, NMDA and P2X receptors have been implicated in diverse chronic and neuropathic pain pathways. HCN1 and 2, primarily expressed in dorsal root ganglion neurons, have received some attention recently as pain mediators. The Na^+/K^+ inward current which flows due to activation of HCN channels (Ih), appears to have a role in mediating neuropathic pain, supported by gain-of-function mutations or over-expression of HCN in animal models. We have systematically tested these diverse ion channels using automated patch clamp. Firstly, we recorded TRPV1 and TRPV3 activated by either ligand or heat. As a number of selective TRPV1 antagonists developed for the treatment of pain show undesirable side effects on core body temperature in clinical studies, drug development targeting this ion channel has been largely halted. Our approach allows antagonists for ligand activation of TRP channels, which do not block the heat activated response, to be identified. Furthermore, we have recorded NMDA receptor combinations NR1/NR2A and NR1/NR2B to investigate both positive and negative modulation. P2X receptors, particularly $P2X_3$ homo- or $P2X_{2/3}$ heteromers, are thought to be involved in pain conditions such as allodynia and hyperalgesia. $P2X_{2/3}$ and $P2X_3$ receptors expressed in CHO or 1321N1 cells were activated by ATP and αß-methylene ATP in a concentration-dependent manner and blocked by suramin or A317491. Lastly, we have focused on the newly identified contribution of Ih to neuropathic pain, by measuring HCN2 currents in HEK cells. We were able to record the current on a high throughput patch clamp instrument. HCN2 was blocked by ivabradine and ZD-7288 in a concentration-dependent manner with an IC_{50} of 5.8 µM (n = 88) and 17.3 µM (n = 93), respectively. Finally, we were able to record Na_V currents and ligand-gated currents mediated by $GABA_A$, AMPA and nAChα7 receptors from glutamatergic-enriched human induced pluripotent

stem cell-derived cortical neurons (hiPSC-neurons) as an excellent model to study neuronal ion channels in a more native environment.

Disclosures: **A.R. Obergrussberger:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **E. Dragicevic:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **N. Becker:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **M. Rapedius:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **T.A. Goetze:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **M.G. Rotordam:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **N. Brinkwirth:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **I. Rinke-Weiß:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **S. Stoelzle-Feix:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **C. Haarmann:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **S. Friis:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **T. Strassmaier:** A. Employment/Salary (full or part-time):; Nanion Technologies Inc. **R. Haedo:** A. Employment/Salary (full or part-time):; Nanion Technologies Inc. **M. George:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **A. Brüggemann:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **N. Fertig:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH.

Poster

218. Somatosensation: Ion Channels

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 218.08/H14

Topic: D.02. Somatosensation

Support: the National Research Foundation of Korea (2011-0018358).

Title: Tentonin 3/TMEM150c expresses in aortic arch and mediates baroreflex function

Authors: H. LU, *J. CHOI, G.-S. HONG, U. OH;
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Abstract: Control of arterial pressure (AP) is an important function that stabilizes ever changing AP. The best known control system is the baroreceptor reflex. Arterial baroreceptors are end organs in the adventitia of carotid sinus or aortic arch detecting AP changes. Mechanosensitive channels are thought to transduce the beat to beat change of AP to electrical activity in big arteries. Some mechanosensitive channels including Piezo1 and 2 were introduced for possible role in baroreceptor function. Previously we also showed that Tentonin 3 (TTN3/TMEM150C), a cation channel activated by mechanical strokes with slow inactivation kinetics, played a role for baroreflex. In this study, we determined further whether TTN3 mediates the baroreceptor

function. In order to see the expression of TTN3 in nerve terminals of aortic depressor nerve, we injected AAV DJ-EF1α-DIO-eYFP into the right nodose ganglion (NG) of *Ttn3-Cre* mouse. The eYFP positive puncta and tortuous fibers were formed a nerve plexus and arborized in aortic depressor nerve terminals along the adventitia of aorta. Previously, we showed that *Ttn3*-/- mice exhibited ambient hypertension, tachycardia, greater fluctuation of APs, and lower baroreflex sensitivity than those of wild-type mice. Using DREADDs system, *Ttn3*-positive neurons in NG were silenced and activated chemogenetically. The chemogenetic silencing or activation of these neurons in NG caused a blood pressure and heart rate increase and decrease, respectively. More importantly, we repeated the rescue experiment on *Ttn3-/-* mice. The overexpression of *Ttn3* in NG of *Ttn3*-/- mice rescued the changes in AP, heart rate, and the baroreflex sensitivity from those observed in $Ttn3^{-/-}$ mice. These results clearly conclude that TTN3 plays an essential role in sensing dynamic AP change in the aorta and carotid sinus.

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Poster

218. Somatosensation: Ion Channels

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GSU Brains & Behavior Seed Grant to DNC
NJH is Brains and Behavior and Honeycutt Fellow
AAP is a 2CI Neurogenomics and Honeycutt Fellow

Title: Mechanisms of cold temperatures rate coding by *Drosophila* CIII neurons

Authors: *N. MAKSYMCHUK, A. SAKURAI, A. A. PATEL, N. J. HIMMEL, D. COX, G. CYMBALYUK;
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Abstract: Animals developed robust mechanisms to protect themselves from noxious cold temperatures which can cause tissue damage and even be fatal. These mechanisms engage all levels, from molecular to behavioral. How neurons encode painful cold stimuli which trigger stereotypical protective behavior is not well understood. *Drosophila melanogaster* is a powerful model organism in which to address cold nociception combining diverse approaches including genetics, calcium imaging, electrophysiology, animal behavior, and computational modeling. *Drosophila* larvae respond to noxious cold by a full-body contraction. Interestingly, this behavioral response is only evoked by a sufficiently fast temperature decrease. Class III (CIII) multidendritic sensory neurons function in noxious cold temperature coding and require the

function of the TRP channels Trpm and Pkd2 for cold-evoked behavior [1].
We compared larval responses to slow and fast temperature changes from 24ºC to the 10ºC. Cold-evoked contraction behavior was potentiated under fast ramping conditions relative to slow. At the fast ramp, CIII neurons showed a definite peak of firing rate and turned silent as the temperature was increased back to 24ºC. At the slow temperature decrease, the firing rate was much smaller. These results suggest that CIII neurons encode rate of temperature change. We suggest that differences in firing rate and behavior could be explained by the inactivation processes of certain TRP channels. We compared the roles of Pkd2 and Trpm currents. Our model shows that the Ca^{2+}-dependence of the inactivation constant of TRP channels could provide a mechanism of observed rate coding. We implemented this mechanism in the computational model, which allowed us to reproduce electrophysiological data - the high peak of firing rate in response to the fast temperature change from 24ºC to 10ºC and silence during temperature return back to ambient levels. When the noxious cold temperature was held constant after fast ramp, Pkd2 channels could inactivate, and low-frequency firing rate could be supported through Trpm, responsible for coding temperature. This is consistent with behavioral data as well. Also, our computational model shows that increased firing rate at fast temperature decline was accompanied by high $[Ca^{2+}]_i$ level, whereas slow ramp resulted in significantly lower $[Ca^{2+}]_i$. We conclude that cooperative action of TRP channels could be responsible for rate coding of noxious cold temperatures and possibly for the coding of the magnitude of temperature change.

References

1. Turner HN et al. Curr Biol. 2016, 26(23), 3116-3128.
2. Maksymchuk N et al. BMC Neurosci, 2018, 19(Suppl 2):64, 8-9.

Disclosures: **N. Maksymchuk:** None. **A. Sakurai:** None. **A.A. Patel:** None. **N.J. Himmel:** None. **D. Cox:** None. **G. Cymbalyuk:** None.

Poster

218. Somatosensation: Ion Channels

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 218.10/H16

Topic: D.03. Somatosensation – Pain

Support: NIH 5 R01 NS091353

Title: $Ca_v3.2$ Ca^{2+} channels modulate nociception in healthy and diabetic mice

Authors: *S. K. SAUER[1], T. HOFFMANN[1], K. KISTNER[2], S. JOKSIMOVIC[3], S. M. TODOROVIC[4], P. W. REEH[5];
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Abstract: Diabetes mellitus causes painful neuropathy (DN) in about 30% of the patients. Recent studies suggest an involvement of glycosylated T-type Ca^{2+} channels ($Ca_V3.2$) in the development of DN. Increased expression and elevated excitability under diabetic conditions have been described. We studied the contribution of $Ca_V3.2$ channels to sensory properties of skin nociceptors in healthy and diabetic C57BL/6 control and $Ca_V3.2$ knockout (KO) mice.
Single fiber recordings showed that in healthy $Ca_V3.2$ KO mice the distribution of nociceptor subpopulations is altered. The prevalence of mechano-cold sensitive cutaneous nociceptors was largely enhanced in comparison to C57BL/6 mice. Diabetes increased the quantitiy of spontaneously active (> 2 spikes/min) neurons from 21% to 57% in C57BL/6 animals. In diabetic $Ca_V3.2$ KO mice there was no such increase. The firing frequency of sponanteously active fibers was increased in C57BL/6 but not $Ca_V3.2$ KO diabetic animals. Application of the T-type channel blocker TTA-P2 (10µM) reduced the spontaneous discharge frequency, supporting the notion that T-type Ca^{2+} channels play a significant role in the development of spontaneous activity of nociceptors in diabetes.
Depolarization of cutaneous nociceptors by 40mM external KCl in healthy C57BL/6 mice induced CGRP release that was independent of $Ca_V3.2$ channels. Skin from STZ-diabetic C57BL/6 mice showed an increase in stimulated CGRP release which was prevented following pre-treatment with Neuraminidase (2u/ml, 2h) and absent in diabetic $Ca_V3.2$ KO mice. In contrast, the CGRP release from isolated sciatic nerves from healthy C57BL/6 mice was reduced by the $Ca_V3.2$ channel blocker TTA-P2 (10µM) or when studied in healthy $Ca_V3.2$ KO animals, indicating a relevant expression and contribution of $Ca_V3.2$ channels along the axon. Only nerves from diabetic C57BL/6 mice showed a reduction of stimulated release.
$Ca_V3.2$ channels contribute to determining sensory properties of nociceptors in healthy mice. Using a mouse model of Type 1 diabetes, we found increased neuronal excitability to which augmented $Ca_V3.2$ channel functions e.g. caused by glycosylation, contributed. Our data underline the role of $Ca_V3.2$ channels in mechanisms leading to painful DN.

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Poster

218. Somatosensation: Ion Channels

Location: Hall A

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Program #/Poster #: 218.11/H17

Topic: D.03. Somatosensation – Pain

Support: NHMRC Grant (1138756)

Title: Piezo2 and STOML3 mediate mechanical excitability of A delta bone marrow nociceptors

Authors: *M. MORGAN, S. NENCINI, J. THAI, J. J. IVANUSIC;
Anat. and Neurosci., The Univ. of Melbourne, Melbourne, Australia

Abstract: Piezo2 is a mechanically activated ion channel that has been shown to play a role in mechanically-induced pain. STOML3 is a protein expressed in microtubules that has been shown to facilitate Piezo2 function. In this study, an *in vivo* electrophysiological bone-nerve preparation was used to record the activity of single A delta-fiber bone marrow nociceptors, in response to a sustained noxious mechanical stimulus (increased intra-osseous pressure), in male Sprague-Dawley rats. Differences in single A delta-fiber unit activity were compared between animals that received knockdown of Piezo2 expression in the DRG by intrathecal injections of Piezo2 antisense oligodeoxynucleotides (n=21 units) and mismatch controls (n= 17 units), or inhibition of STOML3 by marrow cavity application of OB-1, a selective STOML3 inhibitor (n=18 units), compared to vehicle control (n=22 units). There were no differences in the number of units responding to the pressure stimulus in Piezo2 knockdown animals compared to mismatch control animals. However units with a phasic-tonic response to mechanical activation appeared to lose the tonic part of their response after Piezo2 knockdown. Units recorded from Piezo2 knockdown animals also took much longer to recover from stimulus-evoked fatigue than those recorded from control animals that received mismatch oligodeoxynucleotides. Inhibition of STOML3 with OB-1 also caused a significant reduction in single unit activity during sustained pressure. However, it in addition caused reduced activity in the phasic part of the response to applied pressure in some units. Together, these findings suggest that Piezo2 and STOML3 contribute to altered excitability of A delta bone marrow nociceptors and that they might constitute targets for therapeutic benefit in painful conditions driven by mechanical disturbance in bone.

Disclosures: M. Morgan: None. **S. Nencini:** None. **J. Thai:** None. **J.J. Ivanusic:** None.

Poster

218. Somatosensation: Ion Channels

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 218.12/H18

Topic: D.02. Somatosensation

Support: DARPA-GG012363

Title: Piezo2 is an ultrasound-sensitive ion channel that mediates neuromodulation in mammalian peripheral nerve

Authors: *Y. BABA[1], B. U. HOFFMAN[1], C.-K. TONG[1], E. E. KONOFAGOU[2], E. A. LUMPKIN[1];

[1]Physiol. and Cell. Biophysics, [2]Biomed. Engin. & Radiology, Columbia Univ. Med. Ctr., New York, NY

Abstract: Ultrasound enables non-invasive neuromodulation of peripheral and central neurons in the central and peripheral nervous systems. We previously showed that focused ultrasound (FUS) applied to neuronal receptive fields evokes action potentials from sensory neurons in mouse *ex vivo* skin-nerve preparations. Although FUS has been proposed to elicit spikes through temperature change, radiation force, and cavitation, the underlying mechanisms of activation are still unclear. To test these models, we performed electrophysiological recordings from teased nerve fibers in skin-saphenous nerve preparations from adult mice of both sexes. Millisecond FUS pulses to receptive fields in skin reliably excited action potentials, and caused local temperature increases of <0.1ºC. Moreover, FUS thresholds did not differ significantly in $TRPV1^{-/-}$ and littermate control mice. Thus, thermal mechanisms do not mediate FUS-evoked spike firing under these conditions. To assess mechanical mechanisms, we generated $CDX2^{cre}$;$Piezo2^{fl/fl}$ conditional knockout (CKO) mice, which lack the mechantransduction channel Piezo2. Aβ fibers from these mice showed a profound increase in von Frey threshold compared with controls, confirming that Piezo2 is essential for low-threshold mechanical responsiveness (CKO: 13.7mN, control: 0.67mN; n=40 per group; p<0.001, Wilcoxon rank sum test). Similarly, Aβ fibers from CKO mice displayed higher median FUS thresholds for receptive field stimulation compared with controls (CKO: 486 W/cm^2, control: 95 W/cm^2; n=9 & 21; p<0.001). We next compared FUS thresholds between nerve trunk and receptive field stimulation. In Piezo2 control mice, Aβ sensory neurons showed significantly higher FUS thresholds for nerve trunk compared with receptive field stimulation (medians: 515 w/cm^2 in nerve, 109 W/cm^2 in receptive field, n=12; p<0.001). By contrast, Aβ fibers in Piezo2 CKO mice showed comparable FUS thresholds between receptive field (median=307 W/cm^2) and nerve trunk stimulation (median=360 W/cm^2; n=4; p=0.74). Together, our results demonstrate that FUS can activate neural firing through mechanical mechanisms. In particular, our findings identify Piezo2 as a FUS-sensitive mammalian ion channel that transduces receptive field sonication. However, knockout of *Piezo2* did not completely abolish either FUS-evoked or touch-evoked action potentials, suggesting that other biological mechanisms work together with Piezo2 to mediate FUS neuromodulation.

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Poster

218. Somatosensation: Ion Channels

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Program #/Poster #: 218.13/H19

Topic: D.02. Somatosensation

Support: 3 R01 NS055159-08S1
5F31NS100484-02

Title: Regulation of piezo2 currents by gi-protein coupled receptors

Authors: *J. S. DEL ROSARIO, T. ROHACS;
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Abstract: Mechanotransduction is a critical biological process for organisms to discriminate between environmental cues. However, little is known about the molecular and cellular components that contribute to its regulation. Piezo2 channels have been identified as key channels responsible for mechanosensation and mechanical pain. These channels are expressed in Dorsal Root Ganglion (DRG) neurons and genetic mutations in these channels have been shown to impair physiological processes such as light touch, proprioception and balance in humans and mice. Activation of the Gq-coupled bradykinin beta 2 receptor (BDKRB2) have been shown to enhance Piezo2 currents, involving a PKC and cAMP- dependent mechanism. However, whether Gi-coupled receptors in DRG neurons play a role in the regulation of Piezo2 channels is still unexplored. Electrophysiogical experiments in our lab show that activation Gi-protein coupled receptors potentiates Piezo2 currents in DRG neurons and heterologous systems and inhibits Piezo1 currents in HEK293 cells. The potentiation and inhibition of Piezos currents is abolished by blocking Gβγ using the C-terminal domain of beta adrenergic kinase (βARKct). In addition, inhibition of Gβγ-downstream kinases such as mitogen-activated protein kinase (MAPK) or phosphoinositide 3-kinase (PI3K) also abolishes the potentiation of Piezo2 currents by Gi-protein coupled receptors implying an indirect effect of Gβγ on Piezo2 channels. Furthermore, behavioral experiments to assess touch sensitivity show that activation of the Gi-coupled $GABA_B$ receptors enhances mechanosensitivity in female mice, but not in male mice thus suggesting a sexual dimorphism in mice mechanosensitivity. We aim to investigate GPCR signaling in the regulation of mechanoreceptors and dissect specific molecules and proteins that can potentially serve as a basis for the development of new drug targets for the treatment of mechanical-pain.

Disclosures: J.S. Del Rosario: None. **T. Rohacs:** None.

Poster

218. Somatosensation: Ion Channels

Location: Hall A

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Topic: D.03. Somatosensation – Pain

Support: Ministerio de Ciencia, Innovación y Universidades, SAF2016-77233-R co-financed by the European Regional Development Fund (ERDF)

“Severo Ochoa” Program for Centers of Excellence in R&D SEV-2017-0723

Title: Piezo2 channels mediate corneal noxious mechanosensation

Authors: J. FERNÁNDEZ-TRILLO, D. FLOREZ-PAZ, A. IÑIGO PORTUGUÉS, O. GONZÁLEZ-GONZÁLEZ, A. GONZÁLEZ, F. VIANA, C. BELMONTE, ***A. GOMIS**;
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Abstract: The transduction and encoding of mechanical forces is crucial for many of the most important physiological processes of life. Activation of low threshold mechanoreceptors evokes tactile and proprioceptive sensations, while activation of nociceptors evokes painful sensations. Significant progress has been made in understanding the cellular and molecular transduction mechanisms in touch receptors and proprioceptors. In contrast, much less is known about the transduction of mechanical forces by mammalian nociceptors.
Piezo2 is a mechanically-gated ion channel responsible for touch sensation and proprioception. However, mechanosensitivity of skin nociceptors is unaffected in Piezo2-deficient mice. A recent study suggests that Piezo2 is also required for mechanical allodynia, although no consensus has been reached in this aspect of noxious mechanosensation.
The cornea is innervated by two classes of trigeminal mechanosensitive nerve terminals, polymodal nociceptors and pure mechano-nociceptors, whose activation evokes pain. However, the transducing channels underlying their mechanosensitivity have not been identified yet. Thus, the cornea appears a simple and well defined sensory organ to investigate the characteristics of the transducing mechanisms underlying mechanosensitivity in sensory neurons evoking mechanical pain sensations.
In the mouse cornea, double immunostaining of peripheral nerve branches for Piezo2 and TRPV1 revealed fibers that express exclusively Piezo2 and others that co-express Piezo2 with the polymodal nociceptor ion channel TRPV1. Whole-cell patch-clamp recordings, combined with simultaneous mechanical indentation, performed in cultured mouse corneal neurons retrogradely marked with the dye FM1-43 applied onto the cornea, uncovered pure mechanonociceptor and polymodal nociceptor neurons, identified by their response to capsaicin. Both classes of neurons displayed the three well-defined types of mechanically-activated (MA) currents with distinct kinetics: RA, IA and SA currents. Following their electrophysiological characterization, neurons were fixed in situ and processed for Piezo2 immunocytochemistry. We found that Piezo2 is expressed in neurons that displayed the three types of MA currents. Furthermore, functional results examining a constitutive *Piezo2* KO mouse, in which the channel was eliminated exclusively in peripheral sensory neurons, revealed a significant reduction of mechanical responses in different corneal neurons. These findings provide direct evidence for a role of Piezo2 ion channels in mechanotransduction at corneal sensory endings signaling mechanical pain.

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Poster

218. Somatosensation: Ion Channels

Location: Hall A

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Program #/Poster #: 218.15/H21

Topic: D.02. Somatosensation

Title: Piezo1 ion channel is expressed in mouse and human dorsal root ganglion (DRG) neurons

Authors: J. ROH, S.-M. HWANG, C.-K. PARK, ***Y. KIM**;
Physiol., Col. of Medicine, Gachon Univ., Incheon, Korea, Republic of

Abstract: Mechanical stimuli can activate various types of mechanosensitive ion channels (MSCs). Piezo1 and Piezo2 have been identified as mechanosensitive ion channels in the somatosensory system. Previous reports have suggested that Piezo2 plays an important role in recognizing mechanical pain and itch stimuli in dorsal root ganglion (DRG) neurons. A recent study has reported that Piezo1 is expressed in capsaicin-responsive trigeminal ganglion (TG) neurons. This result provides that Piezo1 may be related to orofacial pain pathology. However, very little is known regarding its expression patterns and physiological functions in sensory neurons. Here, we report the expression of Piezo1 *mRNA* and protein in mouse and human DRG neurons. We found that Piezo1 *mRNA* expressed in small-(<25 μm)- and medium-(25-35 μm)-sized mouse DRG neurons. In addition, we confirmed that the physiological responses of Piezo1 activation using Yoda1, a selective Piezo1 agonist. The application of Yoda1 increased the intracellular Ca^{2+}($[Ca^{2+}]_i$) level via extracellular calcium influx and the Yoda1-induced $[Ca^{2+}]_i$ increases were inhibited by GsMTx4, a mechanosensitive ion channel antagonist. To further confirm the specific inhibition of Piezo1, we used an Adeno-associated virus serotype 2/5 (AAV2/5) mediated delivery of short hairpin RNA (shRNA) to mediate efficient Piezo gene silencing. The treatment of AAV2/5 effectively downregulated Piezo1 *mRNA* expression and reduced $[Ca^{2+}]_i$ transients by Yoda1 in DRG neurons. Our results suggest that a previously unrecognized functional expression of Piezo1 in small- and medium-sized DRG sensory neurons. Thus, Piezo1 may have a potential role in the mechanosensitivity regulation of nociceptive neurons.

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Poster

218. Somatosensation: Ion Channels

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Topic: D.03. Somatosensation – Pain

Support: Wolfson Foundation
Wellcome Trust
Arthritis Research UK

Title: A central mechanism of analgesia after Nav1.7 loss-of-function

Authors: *D. I. MACDONALD[1,2], A. P. LUIZ[1], E. C. EMERY[1], R. M. BROWNSTONE[2], S. SIKANDAR[3], J. N. WOOD[1];
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Abstract: Deletion of SCN9A encoding the voltage-gated sodium channel Nav1.7 in sensory neurons of mice, as well as in humans, leads to profound pain insensitivity, but innocuous sensation remains intact. Paradoxically, peripherally-targeted pharmacological antagonists of Nav1.7 have not proven successful as painkillers in clinical trials. This could be linked to endogenous opioid signaling that is enhanced in human and mouse Nav1.7 null mutants but is not activated by Nav1.7 blockers. Importantly, Nav1.7 is expressed at both the peripheral and central terminals of nociceptors. To better understand the mechanisms of analgesia after Nav1.7 loss-of-function, we used a range of optical, electrophysiological and behavioural methods to investigate the effects of Nav1.7 deletion on nociceptor function. As expected, mice lacking Nav1.7 in peripheral sensory neurons were less sensitive to mechanical and thermal pain. By contrast, *in vivo* calcium imaging of dorsal root ganglia using GCaMP showed limited deficits in the responses to noxious mechanical and thermal stimuli. Corroborating this, extracellular recordings of dorsal root ganglia neurons revealed action potential firing evoked by peripheral receptive field stimulation was largely unchanged in Nav1.7 knockout animals. Importantly, although mice lacking Nav1.7 failed to develop heat hyperalgesia after treatment with the inflammatory mediator PGE2, nociceptor somata showed normal levels of PGE2-induced peripheral sensitization. Collectively, these data demonstrate that action potentials evoked by noxious stimuli appear to propagate at least as far as the somata in Nav1.7-deficient nociceptors, indicating that loss-of-function of the channel at the central terminal is the likely locus of analgesia. To test this hypothesis, we examined the effect of Nav1.7 deletion on synaptic transfer from nociceptor central terminals in the dorsal horn using iGluSnFR imaging of glutamate release. In complementary behavioural experiments, we asked whether blocking central terminal opioid receptors was sufficient to reverse Nav1.7-linked analgesia. We found that glutamate

release from afferent terminals in the dorsal horn was compromised, and that analgesia was reversed by central administration of opioid antagonists. Taken together, our findings account for the limited therapeutic utility of peripherally-targeted Nav1.7 blockers and point to a central mechanism of analgesia in NaV1.7 null mutants.

Disclosures: D.I. MacDonald: None. **A.P. Luiz:** None. **E.C. Emery:** None. **R.M. Brownstone:** None. **S. Sikandar:** None. **J.N. Wood:** None.

Poster

218. Somatosensation: Ion Channels

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 218.17/H23

Topic: D.03. Somatosensation – Pain

Title: Dexmedetomidine modulated transient receptor potential ankyrin subtype 1 in mice

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Abstract: Dexmedetomidine, a high-selectivity α2-adrenoceptor(α2-AR) agonists, is widely used as sedative and analgesic agents. However, the mechanism underlying the analgesic effects of dexmedetomidine is unclear. The transient receptor potential ankyrin 1 (TRPA1) is a Ca^{2+} ion channel that mediates nociception through calcium influx of sensory neurons. To investigate the role of the dexmedetomidine, the change of the AITC-induced Ca^{2+} influx after application of dexmedetomidine was tested using Fura-2 based calcium imaging, and the double staining for α2-AR and TRPA1 using immunocytochemistry was performed in dissociated dorsal root ganglion (DRG) neurons. The AITC-induced Ca^{2+} influx decreased after application of dexmedetomidine in DRG neurons. α2-adrenoceptor and TRPA1 are highly co-localized in the DRG neurons whose Ca^{2+} influx was inhibited by dexmedetomidine. These results suggested that inhibition of TRPA1 might be the molecular mechanism underlying analgesic action of dexmedetomidine in peripheral nervous system.

Disclosures: K. Kim: None. **B. Lee:** None. **G. Chung:** None.

Poster

218. Somatosensation: Ion Channels

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Topic: D.03. Somatosensation – Pain

Support: ESTEVE PHARMACEUTICALS SA
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Title: Modulation of the TRPA1 ion channel by sigma-1 receptor

Authors: *E. DE LA PEÑA GARCIA, A. MARCOTTI, J. FERNÁNDEZ-TRILLO, A. GONZÁLEZ, A. GOMIS, F. VIANA;
Inst. de Neurociencias. UMH-CSIC, San Juan de Alicante, Alicante, Spain

Abstract: TRPA1 channels expressed in mammalian nociceptor endings play a critical role in chemonociception as a molecular sensors of reactive irritants, stress and tissue damage. Additionally, TRPA1 has been implicated in noxious cold and mechanical pain sensation. Sigma-1 receptor (σ-1R) is a chaperone mainly located in endoplasmic reticulum membrane, acting as an interoganelle signaling modulator that regulates the trafficking and function of different ionic channels. In the context of pain, pharmacological treatment of mice with the σ-1R antagonist, E-52862 (provided by ESTEVE PHARMACEUTICALS SA), produces antinociceptive effects, and has been shown to reduce the symptoms of neuropathic pain but the molecular mechanism is still unresolved. We studied the possible role of σ-1R in the modulation of TRPA1 channels and the result of this modulation on pain in mice. Measurement of intracellular calcium changes $[Ca^{2+}]_i$ (FURA-2), and patch-clamp recordings were carried out in HEK-293 cells transfected with human TRPA1 (HEK-hTRPA1), and in cultured mouse DRG sensory neurons responsive to 50 µM AITC, a TRPA1 agonist. In both systems, incubation (4-24h) with E-52862 decreased significantly, in a dose dependent manner, the amplitude of $[Ca^{2+}]_i$ responses evoked by AITC. Whole-cell membrane currents in HEK-hTRPA1 cells in response to AITC were also reduced, following incubation with the drug. In DRG sensory neurons the firing of action potentials, recorded in cell-attached mode, the membrane depolarization and the number of action potentials recorded in whole-cell current-clamp configuration evoked by AITC, were also significantly reduced following incubation with E-52862. In mice, we found that pain behaviors (licking, lifting and flickering) associated with TRPA1 activation by intraplantar injection of 10 mM AITC, decreased following intraperitoneal injection 24 h before of (40mg/kg) E-52862. These results implicate TRPA1 channels in the anti-nociceptive effects of σ-

1R antagonists. All experimental procedures were carried out according to Spanish Royal Decree 1201/2005 and the ECC directive 2010/63/EU.

Disclosures: E. De La Peña Garcia: None. **A. Marcotti:** None. **J. Fernández-Trillo:** None. **A. González:** None. **A. Gomis:** None. **F. Viana:** None.

Poster

218. Somatosensation: Ion Channels

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 218.19/H25

Topic: D.02. Somatosensation

Title: Transient receptor potential channel 4 as a novel target to relieve psoriasis

Authors: *S. LEE[1], R. TONELLO[1], Y. CHOI[2], S. JUNG[2], T. BERTA[1];
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Abstract: Psoriasis is a chronic inflammatory skin disease affecting approximately 2% to 3% of the world's population. Psoriasis is characterized by the presence of scaly skin plaques, thickened stratum corneum, infiltration of inflammatory cells, and chronic itch (i.e. pruritus). Although about 60-90% of psoriatic patients have pruritus as the most troublesome symptom, therapeutic options for pruritus are few and current antihistamine drugs are inadequate for the treatment of psoriasis. Transient receptor potential channels are nonselective cationic channels expressed in the brain and sensory ganglia, where they can mediate the transmission of different forms of sensory information, including itch. Of particular interest, we have recently revealed a previously unknown itch signaling pathway in peripheral sensory neurons by which the transient receptor potential channel 4 (TRPC4) mediates itch to serotonergic antidepressants, and most importantly demonstrated the antipruritic effect of a novel TRPC4 inhibitor. Here, we characterize the expression of TRPC4 in peptidergic sensory neurons and show that TRPC4 inhibitor not only alleviates acute itch after serotonin and histamine, but also chronic itch after repeated application of imiquimod (IMQ), a preclinical model of psoriasis. In addition to alleviate itch, intradermal injection of this inhibitor strongly reversed skin inflammation as it significantly reduced IMQ-induced increases in skin scale, erythema, thickness, as well as number of immune cells. Since a TRPC4 inhibitor is already in clinical trial, we expect that this study will rapidly lead to novel and effective clinical treatments for millions of patients suffering from itch and psoriasis.
Keywords: Itch, Psoriasis, Skin inflammation, Sensory Neurons, Transient Receptor Potential channels, TRPC4

Disclosures: S. Lee: None. **R. Tonello:** None. **Y. Choi:** None. **S. Jung:** None. **T. Berta:** None.

Poster

218. Somatosensation: Ion Channels

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Program #/Poster #: 218.20/H26

Topic: D.02. Somatosensation

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Title: Expression of the cold thermoreceptor TRPM8 in rodent peripheral and brain circuits involved in thermal homeostasis

Authors: P. ORDÁS[1], P. HERNÁNDEZ-ORTEGO[1], H. VARA[1], C. FERNÁNDEZ-PEÑA[1], A. REIMÚNDEZ[2], C. MORENILLA[1], A. GUADANO-FERRAZ[3], A. GOMIS[1], M. HOON[4], F. VIANA[1], ***R. SENARIS**[2];
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Abstract: The ion channel TRPM8 is the principal sensor of environmental cold in mammalian sensory nerve endings. It is mainly expressed in a subpopulation of peripheral sensory neurons and little is known about the expression of this ion channel in the brain or the innervation of internal structures by TRPM8 sensory fibers. In the present study we have characterized the expression, anatomical distribution and functionality of TRPM8 channels in rodent central nervous system (CNS) as well as the innervation of relevant thermoregulatory organs using a combination of histological, molecular and electrophysiological techniques.
We determined TRPM8 mRNA by RT-PCR and "*in situ*" hybridization (ISH) in mouse and rat sensory ganglia and brain. Furthermore, GFP immunohistochemistry was carried out in two transgenic TRPM8 reporter mouse models: TRPM8-GFP knock-in mice (*Trpm8*^{EGFPf}) and TRPM8-YFP transgenic mice (*Trpm8*^{BAC}*-EYFP*$^{+}$). Co-expression studies with parvalbumin, calbindin, GABA, CGRP and thyroxin hydroxylase were performed to get further insight into the molecular characteristics of the neurons expressing TRPM8. Finally, we performed patch-clamp recordings of *Trpm8*^{BAC}*-EYFP*$^{+}$ septal neurons.
We demonstrate that TRPM8 is expressed in mouse and rat CNS, although with much lower

levels of expression than in peripheral sensory ganglia. Positive cells were observed in restricted brain areas, especially in the preoptic hypothalamus, septal area, reticular thalamic nucleus and limbic regions with projections widely distributed within the brain and brainstem. Mouse and rat show a similar pattern of expression. We also identified TRPM8 sensory fibers innervating thermoregulatory organs like tail vessels or metabolic organs like the liver. Electrophysiological recordings in brain slices revealed the functionality of this ion channel, but only during combined application of intracellular menthol with external cooling stimuli.
Our findings suggest a role for central and peripheral TRPM8 neurons in autonomic and behavioral thermoregulation. Further experiments are required to fully understand the regulation of this molecular thermosensor within the brain and in internal organs involved in thermoregulatory functions.

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Poster

218. Somatosensation: Ion Channels

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 218.21/H27

Topic: D.03. Somatosensation – Pain

Support: NIH Grant NS055159
NIH Grant GM093290

Title: Gαq sensitizes the TRPM8 ion channel to inhibition by decrease in PIP_2 levels

Authors: L. LIU, Y. YUDIN, J. NAGWEKAR, N. SHIROKOVA, C. KANG, ***T. ROHACS**; Rutgers New Jersey Med. Sch., Newark, NJ

Abstract: The cold and menthol sensitive Transient Receptor Potential Melastatin 8 (TRPM8) channel is important for both physiological temperature detection and cold allodynia. Activation of G-protein coupled receptors (GPCRs) by pro-inflammatory mediators inhibits these channels. It was proposed that this inhibition proceeds via direct binding of $G_{\alpha q}$ to the channel. TRPM8 requires the plasma membrane phospholipid phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$ or PIP_2] for activity. It was claimed however that a decrease in cellular levels of this lipid upon receptor activation does not contribute to channel inhibition. Here we show that supplementing the whole cell patch pipette with $PI(4,5)P_2$ reduced inhibition of TRPM8 by activation of $G_{\alpha q}$-coupled receptors in dorsal root ganglion (DRG) neurons. Stimulating the same receptors activated Phospholipase C (PLC) and decreased plasma membrane $PI(4,5)P_2$ levels in these neurons. $PI(4,5)P_2$ also reduced inhibition of TRPM8 by activation of heterologously expressed

$G_{\alpha q}$-coupled muscarinic M1 receptors. Co-expression of a constitutively active $G_{\alpha q}$ protein that does not couple to PLC inhibited TRPM8 activity, and in cells expressing this protein decreasing $PI(4,5)P_2$ levels using a voltage sensitive 5'-phosphatase induced a stronger inhibition of TRPM8 activity than in control cells. Our data indicate that upon GPCR activation, $G_{\alpha q}$ binding reduces the apparent affinity of TRPM8 for $PI(4,5)P_2$ and thus sensitizes the channel to inhibition induced by decreasing $PI(4,5)P_2$ levels.

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Poster

218. Somatosensation: Ion Channels

Location: Hall A

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Program #/Poster #: 218.22/H28

Topic: D.03. Somatosensation – Pain

Support: NIH Grant RO1 NS031680
JSMEF Data Acquisition Grant CON22735

Title: Maintenance mechanism of nociplastic pain in males

Authors: *K. E. MCDONOUGH[1], K. M. HANKERD[2], J.-H. LA[2], J. M. CHUNG[3];
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Abstract: Recently, the International Association for the Study of Pain defined a third form of pain: *nociplastic pain*. A key mechanism of nociplastic pain is central sensitization (CS) persistently maintained in the absence of an underlying persistent injury. We developed a novel mouse model of nociplastic pain, which uses hindpaw capsaicin injection as a transient injury, followed by innocuous vibration stimulation, making CS persist beyond the normal resolution time. Our lab has previously shown that the normally resolving transient CS by capsaicin is maintained by ongoing nerve activity at the injury site in female mice. We preliminarily found that the persistent CS in our male nociplastic pain model is maintained by different mechanisms. Based on the literature that spinal microglia and their inflammatory mediators play a key role in other models of chronic pain, specifically in males, we hypothesize that nociplastic pain in males is due to CS maintained by activated microglia and subsequent release of inflammatory mediators such as prostaglandins generated through the cyclooxygenase (COX) pathway. To test this hypothesis, spinal microglia and COX were inhibited by intrathecally injecting the microglia-targeting toxin Mac-1-saporin or the COX inhibitor indomethacin, respectively, following establishment of a nociplastic pain state. Secondary mechanical hypersensitivity, a behavioral biomarker of CS, was mitigated by intrathecal Mac-1-saporin, whereas intrathecal

indomethacin had no effect. Our results suggest that spinal microglia maintain persistent CS driving nociplastic pain in males. However, prostaglandins generated through the COX pathway do not seem to be the main inflammatory mediator involved in the maintenance of this CS.

Disclosures: K.E. McDonough: None. **K.M. Hankerd:** None. **J. La:** None. **J.M. Chung:** None.

Poster

218. Somatosensation: Ion Channels

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Topic: D.03. Somatosensation – Pain

Support: NIH Grant R01 NS031680
JSMEF Data Acquisition Grant CON22735

Title: Estrogen mediates peripherally-maintained persistent central sensitization in nociplastic pain

Authors: *K. M. HANKERD, J.-H. LA, J. M. CHUNG;
Dept. of Neuroscience, Cell Biology, and Anat., Univ. of Texas Med. Br., Galveston, TX

Abstract: Nociplastic pain conditions, which disproportionately affect women, are characterized by persistent pain despite resolution of the triggering injury, such as soft tissue injury. Although central sensitization, in which plastic changes in central nociceptive neurons produce an augmented response to afferent input, has been suggested as a key driver of nociplastic pain, the mechanisms underlying how an acute injury-induced, normally resolving central sensitization becomes persistent to result in nociplastic pain are unclear. Using a novel nociplastic pain model characterized by persistent central sensitization, we found that silencing of afferents at the previous injury site attenuated the behavioral biomarker of central sensitization, namely secondary mechanical hypersensitivity, in female C57BL/6N mice, but not in males.
We thus hypothesized that the presence of female hormones, particularly estrogen, are critical for the development of "peripherally-maintained" persistent central sensitization. In ovariectomized females, silencing of afferents at the previous injury site failed to attenuate secondary mechanical hypersensitivity.
However, when ovariectomized females were implanted with mini-pumps containing 17β-estradiol prior to our manipulation producing nociplastic pain state, their secondary mechanical hypersensitivity was attenuated by bupivacaine injection at the previous injury site. Ongoing behavioral and electrophysiological studies characterizing the afferents at the previous injury site suggest that, in females, Transient Receptor Potential channel A1-expressing ($TRPA1^{+}$) afferents are sensitized and that a greater proportion of mechano-sensitive C-fibers at the previous injury

site are spontaneously active, respectively. These results suggest that the presence of estrogen is critical for the sensitization of $TRPA1^+$ mechano-nociceptors, which maintain the peripherally-maintained persistent central sensitization observed in females.
This work was supported by NIH grant R01 NS031680 and JSMEF Data Acquisition Grant CON22735

Disclosures: K.M. Hankerd: None. **J. La:** None. **J.M. Chung:** None.

Poster

218. Somatosensation: Ion Channels

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 218.24/H30

Topic: D.03. Somatosensation – Pain

Support: Apoyo a Proyectos de Investigación e Innovación Tecnológica (DGAPA-PAPIIT) Grant IA202717
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Title: Regulation of pain through the TRPV1 channel by the sigma-1 receptor and progesterone

Authors: *R. JUÁREZ-CONTRERAS[1], M. ORTÍZ-RENTERÍA[1], R. GONZÁLEZ-RAMÍREZ[3], L. D. ISLAS[4], I. LLORENTE[2], S. A. SIMON[5], M. HIRIART[1], T. ROSENBAUM[1], S. L. MORALES-LÁZARO[1];
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Abstract: Nociceptors are specialized neurons that detect adverse environments through activation of molecular players as ion channels. Among them, the Transient Receptor Potential Vanilloid 1 (TRPV1) is an essential non-selective cation channel activated by chemical, mechanical and thermal stimuli. Its function is to transduce the noxious stimuli into electrical signals that the brain can interpret as itching, burning or pain. One of the main fields of study in analgesia research is the regulation of the function/expression of TRPV1 and its interaction with other proteins. In particular, the modulation of this channel by chaperones has poorly explored.

An important chaperone that has also been implied in pain is the Sigma-1 Receptor (Sig-1R), a unique chaperone that can be regulated by ligands, such as progesterone. In this work, we showed that TRPV1 interacts directly with Sig-1R and this molecular association can regulate pain.
Using biochemical, electrophysiological and animal behavior tests, we showed that the antagonism of Sig-1R by progesterone or BD1063 results in the down-regulation of the expression of TRPV1 and the decrease in capsaicin-evocated currents. We also found a reduction in the nociceptive response to capsaicin in male mice treated with the synthetic Sig-1R antagonist BD1063. Moreover, pregnant female mice, showed higher pain threshold to capsaicin than unpregnant mice, suggesting that the high levels of progesterone are protecting to pain produce through the activation of the TRPV1 ion channel.
This study provides a molecular explanation for how Sig-1R and progesterone can regulate the expression of an important receptor to noxious stimuli and lead to a decrease in pain thresholds under certain physiological conditions, like pregnancy.

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Poster

218. Somatosensation: Ion Channels

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 218.25/H31

Topic: D.03. Somatosensation – Pain

Support: NIH Grant GM124055

Title: Effect of TRPA1 antagonist AMG0902 in a rat model for postoperative pain

Authors: *S. E. BEYER[1], D. SUGIYAMA[1], S. G. LEHTO[2], S. KANG[3];
[1]Univ. of Iowa, Iowa City, IA; [2]Neurosci., Amgen Inc., Thousand Oaks, CA; [3]Univ. of Iowa Hosp. and Clinics, Iowa City, IA

Abstract: We have shown previously that reactive oxygen species, such as hydrogen peroxide (H_2O_2), are increased after incision in the wound environment (1), and H_2O_2 increases intracellular Ca^{2+} in DRG neurons *in vitro* via the TRPA1 receptor (2). These findings suggest that sustained pain-related behaviors and increased neuronal activity in nociceptive pathway after muscle injury may be in part mediated by TRPA1 in deep tissue. In this study, we tested the effects of pharmacological blockade of TRPA1 using AMG0902 on *in vivo* nociceptive neural activity after deep muscle incision and after injection of H_2O_2 into the muscle. We also evaluated the effect of AMG0902 on guarding behavior after plantar incision. Adult male and female SD

rats (200 - 300 g) were used. Sample sizes were estimated to achieve 80% power to detect a difference of 40% with an estimated SD of 30 and α of 0.05. The experimenters were blinded to the treatment group. We examined the effect of AMG0902 on the activity of dorsal horn neurons (DHNs). Spontaneously active DHNs were recorded 1 day after gastrocnemius incision. After recording baseline, I.V. AMG0902 (5 mg/kg) or vehicle (DMSO) was injected, and neural activity was recorded. DHN activity after injection of AMG0902 (1.9 ± 2.4; mean ± SD) was lower than the baseline (4.5 ± 3.0; $p < 0.001$). On the other hand, neuronal activity was not different before (4.4 ± 4.0) *vs*. after (4.7 ± 4.7) injection of vehicle. Next, the effect of AM0902 on H_2O_2-induced spontaneous activity of DHNs was evaluated. Neuronal activity was recorded after H_2O_2 injection into the gastrocnemius muscle. Then neuronal activity after administration of AMG0902 (I.V.) or vehicle was recorded. DHNs showed lower neuronal activity after injection of AMG0902 (median 0.74), compared with those in the vehicle group (median 0.06; $p < 0.05$). Lastly, the effect of AMG0902 was examined in the rat plantar incision model. The effect of AMG0902 alone (P.O.), morphine alone (S.C.), and their combination on guarding pain behavior was tested in separate sets of rats. Guarding behavior was significantly reduced by 300 mg/kg, but not by 100 mg/kg, of AMG0902. For morphine, 1 mg/kg, but not 0.03 - 0.3 mg/kg, significantly reduced guarding behavior. When P.O. AMG0902 (100 mg/kg) was added to S.C. morphine (0.3 mg/kg), guarding score was significantly lower compared with the control groups. In conclusion, AMG0902 significantly reduced the spontaneous activity of DHNs induced by incision or by injection of H_2O_2 into muscle. AMG0902 showed an opioid-sparing effect reducing guarding behavior in the plantar incision model. References 1. Sugiyama et al., PLoS One 12:e0170410 2. Sugiyama et al., Anesthesiology 127:695-708, 2017

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Poster

218. Somatosensation: Ion Channels

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 218.26/H32

Topic: B.04. Ion Channels

Title: Probing capsaicin-TRPA1 interactions with computational analysis

Authors: *E. MEDINA-GURROLA[1], R. SYRLYBAEVA[2], M. R. TALIPOV[2], E. E. SERRANO[1];
[1]Biol., [2]Chem. and Biochem., New Mexico State Univ., Las Cruces, NM

Abstract: Capsaicinoids are a structurally related group of alkaloid plant secondary metabolites that confer a pungent taste to chilis. In addition to capsaicin, many other capsaicin-like

compounds have been found to contribute to chili pungency. Capsaicin is a recognized agonist of the transient receptor potential vanilloid 1 channel (TRPV1) and recent findings support a capacity for activation of the transient receptor potential ankyrin1 (TRPA1) channel. Capsaicin is used as a topical analgesic treatment because of its ability to produce sensory desensitization. While the effect of capsaicin has been studied on multiple TRP channels, comparisons of the binding affinity of capsaicin and its analogs with TRP channels are few and their affinity for TRPA1, in particular, is not well characterized. Like TRPV1, TRPA1 is widely distributed throughout the body and can be detected in cells with diverse functions such as nociceptive neurons, mechanosensory hair cells, keratinocytes, epithelial cells of the airways and lung, and cells of the gastrointestinal tract. We are interested in the potential of TRPA1 to serve as a therapeutic target for pain and other disorders and implemented molecular docking as a tool to explore the binding affinity of TRPA1 for different capsaicinoids. Analysis was undertaken with the TRPA1 ion channel structure as determined by electron cryo-microscopy (PDB: 3J9P; Protein Data Bank; https://www.rcsb.org/) and nine capsaicinoids (PubChem; https://pubchem.ncbi.nlm.nih.gov/#query=capsaicin&tab=compound). Capsaicinoid ligands were modeled as flexible molecules while TRPA1 was assigned a rigid structure in its activated conformation. Analysis probed interactions with one subunit of the TRPA1 homotetramer and the binding energy with each ligand was calculated using publicly available tools (UCSF Chimera visualization environment; AutoDock Vina molecular modeling simulation software). When docking was performed with a 100% exhaustive search with three replications, zucapsaicin emerged as the highest affinity ligand in the capsaicinoid family series. Results suggest that molecular simulation studies may help guide the selection and design of capsaicin derivatives for therapeutic purposes and will inform the design of experiments that evaluate TRPA1 responses to capsaicinoids in cells of the nervous system.

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Poster

218. Somatosensation: Ion Channels

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 218.27/H33

Topic: F.03. Neuroendocrine Processes

Title: Capsaicin reduces the hypertrophy of uterus Hartley guinea pigs induced with polycystic ovary syndrome

Authors: *V. ALATRISTE, C. A. ZERÓN-ALVARADO, D. LIMÓN, L. MARTÍNEZ-MENDIETA, I. MARTÍNEZ-GARCÍA, F. LUNA;
BUAP, Puebla, Mexico

Abstract: The polycystic ovary syndrome (PCOS) is a pathology that presents endocrine, physiological and behavioral alterations. In women there is hypertrophy of the uterus and decreases fertility. Our objective was study the role of the capsaicin on the cell development of uterus of Hartley guinea pigs with experimental induced PCOS. We used 4 groups (n=4), intact guinea pigs (control), vehicle (I.S.S.), letrozole (1 nM), and letrozole+capsaicin (cap+let) (each 1nM). At the P10 we administrated orally 1mL of I.S.S. for 40 days (vehicle), and letrozole 1 nM in 1 mL also for 40 days orally (letrozole 1 nM). And for let+cap, we gave the letrozole as previously described, and at P51 we adminstrated the capsaicin (1nM in 100 μL of I.S.S.) subcutaneous in dorsal region for 15 days. Then we evaluated 3 estrous cycles and sacrificed the animals by CO_2 exposure and then we obtained the uterus The uterus were fixed, and histological treated to obtain 5 μm slides by microtome. Then some slides were stained with hematoxylin and eosin to determinate the changes in the different epitheliums, other slides were stained with Mason Trichromic to evaluate the connective tissue and finally we located the TRPV1 in the uterus by immunohistochemestry (IHC). All studies were performed with Motic and ImageJ program. We obtain thatl letrozle animals increases the thickness of endometrium, myometrium and ciliary epithelium vs control and vehicle. With let+cap we got than the thickness of endometrium, myometrium and ciliary epithelium vs control and vehicle decreases. By IHC, we saw differences in the expression of TRPV1 in letrozole and let+cap treared guinea pigs. In conclusion the capsaicin decreases the hypertrophy in the uterus in guinea pigs with PCOS, however more studies must be perform to test more doses and function of uterus.

Disclosures: V. Alatriste: None. **C.A. Zerón-Alvarado:** None. **D. Limón:** None. **L. Martínez-Mendieta:** None. **I. Martínez-García:** None. **F. Luna:** None.

Poster

219. Somatosensation: Pain Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 219.01/H34

Topic: D.03. Somatosensation – Pain

Support: Grant-in-Aid for Early-Career Scientists

Title: Formalin-induced nociceptive response is enhanced by serum exosomes isolated from partial sciatic nerve ligation (PSL) mice

Authors: *K. HAMAMURA[1], S. KATSUYAMA[2], T. KOMATSU[1], T. SAKURADA[1], K. ARITAKE[1];
[1]Daiichi Univ. of Pharm., Fukuoka, Japan; [2]Tokyo Univ. of Pharm. and Life Sci., Tokyo, Japan

Abstract: [Aim of Investigation]Exosomes are small (40-150 nm) membrane vesicles of endocytic origin that are found in bodying fluids, and supporting their role in intercellular

communication. Although recent studies have demonstrated that various biomarkers involved in the extent of pain from the serum exosomes (Adv Pharmacol. 75: 35-62, 2016), the effects of exosomes on the onset and progression of pain have not been elucidated. The objective of this study was to examine the effects of serum exosomes in mice with PSL on nociceptive responses induced by 0.5% formalin.
[Methods]Ligation of partial sciatic nerve was performed in mice according to the procedure described by our previous report (Molecular Pain, 6: 83-90, 2010). For sham-operation, the sciatic nerve was exposed, but not manipulated. Exosomal fraction in serum on day 7 post-PSL was isolated by 2 steps; ExoQuick® and EVSecond L70®, were used according to the manufacturer's instructions. Exosomes were administered by intrathecal (i.t.) route at the level of the 5^{th} or 6^{th} vertebrae. 0.5% formalin was injected into the plantar surface of the right hindpaw (s.c.). Each mouse was immediately returned to the observation chamber after formalin injection. Licking and biting of the injected hindpaw were defined as a nociceptive response, and the summation of time spent licking and biting was measured for the first 5 min after a low concentration of formalin.
[Results and Conclusions]We have confirmed that the i.t. injection of serum exosomes from PSL mice or sham-operated mice transferred into the normal mice did not show any spontaneous nociceptive responses. However, 0.5% formalin-induced nociceptive response was significantly enhanced by i.t. pretreatment with serum exosomes isolated from PSL mice but not from sham mice. In addition, we digested the exosomes isolated from PSL with trypsin to obtain the ‘‘surface protein shaved" exosomes. The surface protein shaved PSL exosomes were ineffective on formalin-induced response.
Our data indicate that the surface protein of exosomes in mice with PSL may play an important role in enhancing nociceptive responses.

Disclosures: K. Hamamura: None. **S. Katsuyama:** None. **T. Komatsu:** None. **T. Sakurada:** None. **K. Aritake:** None.

Poster

219. Somatosensation: Pain Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 219.02/H35

Topic: D.03. Somatosensation – Pain

Support: NIH K99DE028019-01

Title: Sex differences in TRPV1 expression, activation and associated behavior in two mouse models of oral cancer

Authors: *N. N. SCHEFF, H. WILLIAMS, A. BHATTACHARYA, B. L. SCHMIDT; Bluestone Ctr. for Clin. Res., New York Univ., New York, NY

Abstract: We have reported sex differences in oral cancer patients and mouse models of oral cancer pain. We sought to determine the contribution of TRPV1 to sex differences in oral cancer pain. We used two oral cancer mouse models: the 4-nitroquinoline-1-oxide (4NQO) oral carcinogenesis model and the tongue xenograft model created by inoculating a human oral SCC cell line (HSC-3) into immunocompromised mice. We measured TRPV1 expression and activation in anatomically relevant trigeminal (TG) neurons innervating the site of tongue cancer. TG neurons were labeled with the retrograde tracer, DiI, two weeks prior to 4NQO treatment or HSC-3 inoculation. We measured TRPV1-mediated behavioral sensitivity to agonist, capsaicin, with a two-bottle (capsaicin versus vehicle) drinking choice assay. At the completion of *in vivo* experiments, TG neurons were harvested and fixed for immunohistochemistry or mechanically dissociated. TRPV1 protein expression was measured using immunohistochemistry in TG sections from HSC-3 xenograft and sham mice. TRPV1 responsiveness to capsaicin in dissociated TG neurons was measured with Ca^{2+}imaging in 4NQO and vehicle-treated mice. We found behavioral sensitivity to 1μm capsaicin in the drinking water in female mice with HSC-3 cancers starting at post-inoculation day 7 and continuing to post-inoculation day 31. Male mice with HSC-3 cancers did not demonstrate significant capsaicin sensitivity compared to sham mice at any time point. We found a significant increase in TRPV1-immunoreactivity in DiI+ TG neurons in female mice with HSC-3 cancers (70.1±3.9%) compared to male mice (55.8±3.0%). We found that 83.9±3.2% of DiI+ TG neurons from female mice with 4NQO-induced oral SCC were capsaicin-responsive compared to 60.4±5.4% in male mice. There was no significant difference in the number of DiI+ capsaicin responsive neurons in vehicle-treated male and female mice. These data suggest that there are sex differences in TRPV1 expression, activation and sensitization in oral cancer.

Disclosures: N.N. Scheff: None. **H. Williams:** None. **A. Bhattacharya:** None. **B.L. Schmidt:** None.

Poster

219. Somatosensation: Pain Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 219.03/H36

Topic: D.03. Somatosensation – Pain

Support: JSPS KAKENHI

Title: GRK2 contributes to the resolution of acute pain by controlling protein phosphorylation in the DRG neurons

Authors: *H. TAKEMURA, F. AMAYA;
Kyoto Prefectural Univ. of Med., Kyoto, Japan

Abstract: Reduced activity of G protein-coupled receptor kinase 2 (GRK2) in the DRG neuron is associated with the development of chronic pain. We investigated the role of GRK2 in the resolution of acute pain hypersensitivity after the plantar incision. Kyoto Prefectural University of Medicine Animal Care Committee approved all experimental procedures. Experiments were performed according to the guidelines of the NIH. Male S-D rats (200g) were used in this study. Rats received plantar incision under isoflurane anesthesia. GRK2 inhibitor or vehicle were injected intraperitoneally 7 days after the plantar incision. Behavioral experiment with von Frey hair and radiant heat was performed to determine mechanical and thermal pain threshold for 3 days after the injection. Separately, L4 and L5 DRG were obtained from rats after the inhibitor treatment. GRK2 expression in the DRG was analyzed by the immunohistochemistry and western blotting. Phosphorylated- and whole- protein signals were visualized after 2-dimensional SDS PAGE. Phosphorylation level was compared between GKR2 inhibitor treated- and vehicle treated- group to identify proteins underwent dephosphorylation by GRK2 inhibitor treatment.Plantar incision induced acute pain hypersensitivity against thermal and mechanical stimulation. Pain hypersensitivity was returned nearly to the baseline level 7 days after the incision. Systemic GRK2 inhibitor treatment at this period produced relapse of the mechanical, but not thermal pain hypersensitivity continued for 3 days. GRK2 inhibitor did not affect on the behavioral threshold of the naive rats. GRK2 expression was significantly increased in the DRG neurons 7 days after the incision. Mass spectrograph identified 14 proteins that underwent dephosphorylation by GRK2 inhibitor.These results demonstrated that up-regulation of GRK2 expression contributes to the resolution of acute pain by controlling protein phosphorylation in the DRG neurons.

Disclosures: H. Takemura: None. **F. Amaya:** None.

Poster

219. Somatosensation: Pain Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 219.04/H37

Topic: D.03. Somatosensation – Pain

Support: NIH grant NS065926

Title: Type 1 interferons act directly on nociceptors to produce pain sensitization: Implications for viral infection-induced pain

Authors: *P. BARRAGAN-IGLESIAS, U. FRANCO-ENZASTIGA, A. WANGZHOU, T. PRICE;
Sch. of Behavioral and Brain Sci., Univ. of Texas At Dallas, Richardson, TX

Abstract: One of the first signs of viral infection is body-wide aches and pain. While this type of pain usually subsides, at their extreme, viral infections can cause painful neuropathies that can last for decades. Neither of these types of pain sensitization are understood. It is known that viral stimulation produces interferons (IFNs), which, in turn, activate their specific receptors resulting in downstream activation of a variety of signaling pathways. We tested the hypothesis that viral infection produces type I interferons that then activate receptors for this immune molecule that are expressed on dorsal root ganglion (DRG) neurons to cause pain sensitization. Incubation of cultured DRG neurons with interferon α (IFN-α, 300 U/mL) and Interferon β (IFN-β, 300 U/mL) produced a rapid increase in the phosphorylation of the JAK-STAT signaling pathway involving p-JAK1, p-STAT1, STAT1, and p-STAT3. We also observed robust activation of p-ERK, p-AKT and the subsequent phosphorylation of eIF4E at serine 209. We did not observe changes in mTOR or RS6 phosphorylation. Unexpectedly, IFN stimulation of cultured DRG neurons did not produce any significant changes in signaling components of the integrated stress response (ISR) such as BIP, p-eIF2α or p-PKR after 1h-6h of IFNs exposure. Using immunofluorescence in vitro, an increase in p-eIF4E^{S209} in small/medium diameter neurons was detected after 1hr treatment suggesting that type I IFNs exerts their actions directly on neurons. In support of this, single cell data analysis of small/medium diameter neurons from the DRG identified that both mRNAs for Interferon receptor 1 (Ifnar1) and 2 (Ifnar2) are broadly expressed in primary sensory neurons with some differences mainly for Ifnar2 which is more abundant in a subgroup of neurons that express Nppb (natriuretic peptide B) and F2rl1 (Proteinase-Activated Receptor 2). Intraplantar (i.pl.) injection of IFN-α (300 U/25uL) and Interferon β (IFN-β, 300 U/25 uL) in mice induced mechanical, but not thermal, hypersensitivity with no observable sex-differences. Mechanical hypersensitivity was partially attenuated in mice lacking the eIF4E phosphorylation at serine 209 (p-eIF4E^{S209A}). Moreover, similar results were obtained in animals lacking MNK1 (MNK1$^{-/-}$), the specific kinase that phosphorylates eIF4E. Our results suggest that MNK1-eIF4E signaling pathway mediates peripherally-mediated nociceptive actions of type I interferons and that these signaling events could be involved in the mechanisms that produce viral-evoked pain responses.

Disclosures: **P. Barragan-Iglesias:** None. **U. Franco-Enzastiga:** None. **A. Wangzhou:** None. **T. Price:** None.

Poster

219. Somatosensation: Pain Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 219.05/H38

Topic: D.03. Somatosensation – Pain

Support: NIH Grant NS065926 (TJP)
NIH Grant NS102161 (TJP and ANA)

Title: Investigation of sex-dependent differentially translated mRNAs in nociceptors

Authors: *D. TAVARES-FERREIRA[1], P. R. RAY[1], A. WANGZHOU[1], I. SANKARANARAYANAN[1], S. SHIERS[1], P. BARRAGAN-IGLESIAS[1], A. N. AKOPIAN[2], T. J. PRICE[1];
[1]Sch. of Behavioral and Brain Sci., Univ. of Texas at Dallas, Richardson, TX; [2]Dept. of Endodontics, UT Hlth. San Antonio, San Antonio, TX

Abstract: Chronic pain is a complex disease that is estimated to affect almost 100 million Americans with a great impact on their everyday quality of life. Evidence clearly points towards how chronic pain is disproportionately more frequently reported in women than in men. However, the mechanisms that underlie these sex-differences remain largely unknown. There is an increasing interest in understanding those mechanisms to develop more effective, customized therapeutics. The objective of this project is to investigate differentially translation of mRNAs between males and females in dorsal root ganglia (DRG) nociceptors. We used male and female transgenic Nav1.8-Cre-TRAP (translating ribosome affinity purification) mice (aged 8-12 weeks) to address this question. The Nav1.8-Cre mice were crossed with mice that have a floxed L10a-eGFP fusion. Because L10a protein is associated with translating ribosomes, this crossing generates mice with tagged ribosomes exclusively expressed in Nav1.8 positive neurons, most of which are nociceptors. DRGs were dissected, homogenized and incubated with antibodies that specifically target the eGFP tag. Ribosomes were, then, immunopurified and the bound RNA extracted and sequenced. We identified several genes that were differentially translated between male and females in DRG nociceptors. Gene enrichment analysis suggests that the identified genes may be involved in inflammatory response and axon development. *Rpl41* (a ribosomal protein) and *Tsc22d3* (anti-inflammatory protein) were found to have increased translation efficiency in male DRG. One of the identified genes with increased translation efficiency in female DRG was Prostaglandin D2 synthase *(Ptgds*). This protein catalyzes the conversion of prostaglandin H2 to prostaglandin D2 and evidence suggests that it plays a role in the regulation of nociception. Immunohistochemistry experiments confirmed that Ptgds is highly expressed in female DRG neurons compared to male DRG neurons. This study identifies genes that have differential translation efficiency in males versus females DRG nociceptors at the basal level and contributes to a better understanding of how males and females may respond differently to pain and injury.

Disclosures: D. Tavares-Ferreira: None. **P.R. Ray:** None. **A. Wangzhou:** None. **I. Sankaranarayanan:** None. **S. Shiers:** None. **P. Barragan-Iglesias:** None. **A.N. Akopian:** None. **T.J. Price:** None.

Poster

219. Somatosensation: Pain Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 219.06/H39

Topic: D.03. Somatosensation – Pain

Title: Repeated mechanical stimuli at change time intervals have influence on pressure pain thershold

Authors: *S. NAGAHAMA;
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Abstract: Nociceptors in the skin are present in free nerve endings of Aδ fibers and C-fibers. The Aδ fibers develop picking and fast pain in response to mechanical stimulation. It is well known that pain receptor is tonic or slowly adapting and it is generally thought that pain receptor is non-adapting type. There is little report about pain sensory and adaptation mechanism of Aδ fiber, and it is not clear of pain sensory mechanism of Aδ fiber.
The author reported that repeated mechanical stimuli to nociceptor increase pressure pain thresholds, PPTs (SfN 2018). A total of 16 participants took part in the study (ranging from 20 to 22 years; 8 males and 8 females). In 7, 10, 20, 30-seconds intervals the PPTs were significantly increased. In contrast, there were increase tendency of the PPTs in 5-seconds intervals, but there were no significant changes of them. The present study focused on the adaptation of pain sensory by measuring the influence of repeated mechanical pain stimuli at 5-seconds intervals on PPTs. A total of 24 males participants took part in this study. All experiments were done at intervals more than one week. The participant was pricked with the pressure algometer at the same point of right forearm medial cutis. Control was the average of PPTs measured 3 times at the same point. After the measurement of control, the participant was stimulated at the same point 12 times every 5 seconds successively, and measured a change of PPTs. The significance of the difference among samples was determined by a one-way repeated measures ANOVA followed by a Bonferroni multiple test. Differences at $P<0.05$ were termed significant. In the first experiments at 5-second intervals, PPTs s of the 12^{th} stimulus significantly increased. In addition, PPTs were further measured by the similar method 1 hour, 4 hours and 24 hours after the first experiments. Then the control was measured in all cases and PPTs were measured by the similar method at the same point of the participant. A tendency of increase was seen in PTTs of the 6^{th} and the 12^{th} stimulus 24 hours later PPTs of control stimulus increased 4 hours later, compared with the first experiments. These results showed that repeated mechanical stimuli at 5-secomds intervals increased PPTs. It was suggested that Aδ fiber was adapted by the repeated mechanical stimuli at short time intervals.

Disclosures: S. Nagahama: None.

Poster

219. Somatosensation: Pain Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 219.07/H40

Topic: D.03. Somatosensation – Pain

Support: 1R01DE026806-01A1

Title: Legumain induces trigeminal nociceptor hyperexcitability through PAR_2

Authors: *E. W. C. CHEN[1], D. CHO[1], H. WILLIAMS[1], L. EDGINGTON-MITCHELL[2], N. W. BUNNETT[3], B. L. SCHMIDT[1];
[1]New York Univ. Oral Cancer Ctr., New York Univ. Col. of Dent., New York, NY; [2]Univ. of Melbourne, Melbourne, Australia; [3]Dept. of Surgery, Columbia Univ. Col. of Physicians and Surg, New York, NY

Abstract: Legumain (Lgmn) is elevated and activated in human oral squamous cell carcinoma (SCC). Lgmn is an asparaginyl endopeptidase that potentially cleaves protease activated receptor 2 (PAR_2) on primary afferent neurons. We have shown that Lgmn induces orofacial nociception in a rodent model. Lgmn-induced orofacial nociception requires PAR_2 expression on $Na_v1.8$-positive neurons. We investigated whether Lgmn induces hyperexcitability in trigeminal ganglia neurons using whole-cell patch clamp recording.
We measured the rheobase of trigeminal nociceptors (neurons measuring 15 to 20 µm) from C57BL/6J mice following exposure to Lgmn, Lgmn vehicle and Lgmn plus Lgmn inhibitor. Neurons exposed to Lgmn (20 ng/ml, 10 min) exhibited significantly decreased rheobase compared to neurons exposed to Lgmn vehicle (Lgmn vehicle: 110.0 ± 31.4 pA, n=11; Lgmn: 26.9 ± 22.1 pA, n=13, Student's t-test, $P < 0.0001$). To confirm the requirement for protease activity of Lgmn, we pre-incubated Lgmn with the Lgmn inhibitor (10 µM for 10 min). Lgmn inhibitor abolished the hyperexcitability induced by Lgmn. The rheobase for the Lgmn plus Lgmn inhibitor group was no different from the Lgmn vehicle (Lgmn + inhibitor: 94.0 ± 42.4 pA, n=10). To investigate the requirement of PAR_2 for Lgmn-induced hyperexcitability of trigeminal nociceptors, we analyzed Lgmn-induced hyperexcitability in trigeminal ganglia neurons from $Par_2^{-/-}$ mice. Exposure to Lgmn (20 ng/ml, 10 min) failed to induce hyperexcitability in trigeminal neurons from the $Par_2^{-/-}$mice. The rheobase of Lgmn-treated and vehicle-treated trigeminal neurons from $Par_2^{-/-}$ mice was similar (vehicle treated: 65.3 ± 26.0 pA, n=13, Lgmn: 62.0 ± 30.1 pA, n=10). Our results suggest that Lgmn induces hyperexcitability of trigeminal nociceptors through cleavage of PAR_2.

Disclosures: E.W.C. Chen: None. **D. Cho:** None. **H. Williams:** None. **L. Edgington-Mitchell:** None. **N.W. Bunnett:** None. **B.L. Schmidt:** None.

Poster

219. Somatosensation: Pain Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 219.08/H41

Topic: D.03. Somatosensation – Pain

Support: Swedish Research Council
Pain Relief Foundation
ALF Östergötland

Title: The physiology of human A-beta high-threshold mechanoreceptors

Authors: *S. S. NAGI[1], A. G. MARSHALL[2], A. MAKDANI[3], E. JAROCKA[4], F. P. MCGLONE[3], H. OLAUSSON[1];
[1]Linköping Univ., Linköping, Sweden; [2]Univ. of Manchester, Manchester, United Kingdom; [3]Liverpool John Moores Univ., Liverpool, United Kingdom; [4]Umeå Univ., Umeå, Sweden

Abstract: The canonical view is that pain in humans is signaled exclusively by slowly conducting, thinly myelinated (A-delta) and unmyelinated (C) afferents. While other mammals have thickly myelinated, rapidly conducting (A-beta) nociceptors, these have not been demonstrated in humans. Here, we performed single-unit axonal recordings (microneurography) from mechanoreceptive cutaneous afferents in the peroneal and radial nerves of healthy participants. We identified A-beta high-threshold mechanoreceptors (HTMRs) that were insensitive to gentle touch, encoded painful skin indentations, and had conduction velocities similar to A-beta low-threshold mechanoreceptors. Intra-neural electrical stimulation of single A-beta HTMRs evoked sharp and pinprick-like painful percepts. These findings provide evidence that humans, like other mammals, are equipped with fast-conducting nociceptors. The existence of these afferents questions the validity of the dichotomous fast touch-slow pain systems in classical teaching and calls for a reappraisal of neurological views that mechanical pain examination specifically assesses small-fiber function. Further investigations are needed into the detailed response characteristics, ion-channel properties, and spinal and cortical projections of A-beta HTMRs, given the implications for understanding nociception, nocifensive behavior, and clinical pain states.

Disclosures: S.S. Nagi: None. **A.G. Marshall:** None. **A. Makdani:** None. **E. Jarocka:** None. **F.P. McGlone:** None. **H. Olausson:** None.

Poster

219. Somatosensation: Pain Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 219.09/H42

Topic: D.03. Somatosensation – Pain

Support: NS065926

Title: MNK1/2 inhibition by eFT508 prevents IL-6 induced hyperexcitability in DRG nociceptors

Authors: *V. JEEVAKUMAR, A. K. AL-SARDAR, F. MOHAMED, G. DUSSOR, T. PRICE; The Univ. of Texas At Dallas, Richardson, TX

Abstract: Phosphorylation of the 5' cap-binding protein eIF4E by MAPK interacting kinases MNK1/2 is important for nociceptor sensitization and the development of chronic pain (Moy et al., 2017). IL-6 induced DRG nociceptor excitability was attenuated in Mnk1/2$^{-/-}$ mice and by the nonselective MNK1/2 inhibitor cercosporamide. Here, we tested the hypothesis that inhibition of the MNK-eIF4E signaling pathway using the highly selective inhibitor eFT508 will prevent IL-6 induced hyperexcitability in DRG nociceptors. DRGs were cultured from male and female mice, 5-7 weeks old. The treatment groups were: Vehicle 1hr, IL-6 50 ng/ml 1 hr, eFT508 25 nM ~ 15 hrs followed by IL-6 for 1 hr and eFT508 only for ~ 15 hrs. Whole-cell patch clamp recordings were done in small diameter neurons (25-30 pF) to measure spontaneous activity, and membrane excitability in response to ramp depolarization. One hr IL-6 treatment increased spontaneous action potential firing from rest potential compared to vehicle group, and this effect was blocked by pretreatment with eFT508. eFT508 treatment alone did not induce spontaneous firing (Veh n = 14 cells, 1.5 ±0.7 APs; IL-6 n = 10, 10.5±4; eFT508+IL-6 n = 9, 1.4±0.76; eFT508 only n = 6, 5.1±3.6; $F(3,35) = 3.202$, $P = 0.03$, Veh vs IL-6 $P = 0.02$, Dunnett's test). In a second experiment, ramp currents from 100 pA to 700 pA , delta 200 pA, 1000 ms, were injected into the nociceptors held near -60 mV to elicit action potential firing. IL-6 treatment for one hr resulted in higher numbers of action potentials compared to vehicle group at all current steps. Pretreatment with eFT508 blocked IL-6's effect. eFT508 treatment alone did not cause hyperexcitability to ramp currents. There was a main effect of drug treatment ($F(3,35) = 3.38$, $P = 0.02$) with posthoc Dunnett's test revealing significant differences between the Veh and IL-6 groups at all current steps. Basic membrane properties including resting membrane potential, input resistance and rheobase were similar across groups, with the AP latency showing a trend towards a decrease in the IL-6 group (Veh 9.09±0.75 ms, IL-6 6.71±0.52 ms, eFT508 + IL-6 7.54±0.6 ms). In conclusion, our electrophysiology data reveal that increased responsiveness of nociceptors to inflammatory mediators can be attenuated by inhibition of the MNK-eIF4E pathway. Inflammation mediated nociceptor excitability is regulated by membrane trafficking of

voltage-gated channels, and phosphorylation of ligand gated channels. Therefore it would be pertinent to further investigate the mechanisms underlying inflammatory mediators and the influence of eIF4E phosphorylation mediated translation of mRNAs in downstream events involved in nociceptor plasticity.

Disclosures: V. Jeevakumar: None. **A.K. Al-Sardar:** None. **F. Mohamed:** None. **G. Dussor:** None. **T. Price:** None.

Poster

219. Somatosensation: Pain Mechanisms

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Program #/Poster #: 219.10/H43

Topic: D.03. Somatosensation – Pain

Support: NIH Grant NS104964
American Pain Society
Rita Allen Foundation

Title: Involvement of cckergic system on persistent pain and morphine analgesia following chronic inflammation and peripheral nerve injury in mice

Authors: *D. W. FERREIRA[1,2], L. RUELLE-LE GLAUNEC[1,3], C. M. AROKIARAJ[1,2], R. P. SEAL[1,2];
[1]Dept. of Neurobio., [2]Pittsburgh Ctr. for Pain Res., Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA; [3]Life Sci. Dept., Univ. of Strasbourg, Strasbourg, France

Abstract: Cholecystokinin (CCK)-8 is the most abundant neuropeptide in the nervous system and has demonstrated roles in feeding, anxiety, memory and pain. Although past efforts provided clear pharmacological evidence supporting the importance of the CCKergic system in persistent pain and morphine analgesia at the spinal level, many fundamental circuit-based questions about CCK and its receptors, CCK1 and CCK2, remain, most critically, what are the cellular sources of CCK and what neurons are involved in mediating the actions of the receptors? Here we report that intrathecal (i.t.) injection of CCK2R antagonist CI-988, but not CCK1R antagonist SR27897 reverses mechanical allodynia induced by CFA on day 5 (D5) and SNI on day 7 (D7) in mice. In naive conditions, CCK and CCK2R gene expression are significantly higher in SC than DRG, while CCK1R is higher in DRG than in spinal cord. In DRG, CCK2R gene expression is dramatically increased (~60-fold) following SNI D7, whereas a small increase in CCK2R gene expression (~2-fold) and a decrease in CCK1R gene expression (~25%) are found after CFA D5. Nevertheless, at a later chronic stage of SNI (8 weeks), the DRG levels of CCK2R gene expression are still elevated (~42-fold) and CCK2R antagonist (i.t.) significantly reverses mechanical allodynia. We also tested the effect of spinally delivered CCK receptor antagonists

on morphine analgesia after CFA or SNI. CCK2R antagonist (i.t.) enhances the effects of subcutaneous (s.c.) morphine following CFA D5 and SNI D7, whereas CCK1R antagonist (i.t.) enhances the effect of s.c. morphine on CFA, but not on SNI. Interestingly, a decrease in DRG levels of mu opioid receptor (Oprm1) (~40%) and delta opioid receptor (Oprd1) (~30%) gene expression are observed after SNI, but not after CFA. In DRG from both naïve and SNI mice, CCK1R is expressed by the TH^+ population and by myelinated neurons including a $TrkC^+$ population that expresses PV and $N52^+$, but not by $IB4^+$, $TRPV1^+$, $CGRP^+$ and SP^+ neurons. In DRG from SNI, but not in naïve mice, CCK2R is expressed by $N52^+$ and $TrkC^+/PV^-$ populations as well as a large number of unmyelinated neurons that surprisingly lack all of the common markers, potential due to SNI induced down-regulation. Finally, DRG from SNI mice show that CCK1R and CCK2R are expressed by $Oprd1^+$, but not $Oprm1^+$ neurons. Taken together, our results show that CCK2R blockade prevents mechanical allodynia and CCK-mediated antagonism of morphine analgesia, possibly via the delta opioid receptor. Identifying and understanding the spinal level circuits that require CCK-signaling in mechanical allodynia and morphine analgesia will provide new opportunities to develop more effective, non-addictive therapies.

Disclosures: D.W. Ferreira: None. **L. Ruelle-le Glaunec:** None. **C.M. Arokiaraj:** None. **R.P. Seal:** None.

Poster

219. Somatosensation: Pain Mechanisms

Location: Hall A

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Program #/Poster #: 219.11/H44

Topic: D.03. Somatosensation – Pain

Support: NIH grant DE018661
NIH grant DE023090

Title: Characterization of membrane mechanics of acutely dissociated dorsal root ganglion neurons of rats by the laser tweezer optical trapping technique

Authors: *J. GU, F. EROL, S. TONOMURA;
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Abstract: Membrane mechanics such as plasma membrane tension and membrane stiffness are important biophysical properties of cells that have profound impact on cell functions under both physiological and pathological conditions. Somatosensory neuron membrane mechanics play a role in mechanotransduction for the sense of touch and mechanical pain. In the present study, we used dorsal root ganglion (DRG) neurons acutely dissociated from rats to study their membrane mechanics by using the laser tweezer optical trapping technique. DRG neurons used in the

present study had cell sizes of 28.95 ± 1 μm (n = 39) in diameters. Membrane tethers were pulled from these neurons with a silicon bead optically trapped by infrared laser beam. The forces for holding the membrane tethers by optically trapped beads (F_0) in the acutely dissociated DRG neurons were 26.586 ± 3 pN (n = 19). The radii of membrane tethers determined by the fluorescent densitometry method were 42.63 ± 5 μm (n = 19). These two measures yielded membrane tension of 56.24 ± 7 pN/μm (n = 19) and membrane stiffness of 0.19 ± 0.03 pN*μm (n = 19) in these acutely dissociated DRG neurons. Following the treatment of these DRG neurons with 10 μM cytochalasin D (CD) for 30 min, membrane tension of these neurons was significantly reduced to 35.15 ± 5 pN/μm (n = 16), but membrane stiffness (0.20 ± 0.04 pN*μm, n = 16) was not significantly affected. Collectively, these findings suggest that the laser tweezer optical trapping technique may be used in future to explore membrane mechanics of DRG neurons following *in vivo* pathological conditions such as tissue inflammation and nerve injury. This study was supported by NIH grants DE018661 and DE023090 to J.G.G.

Disclosures: J. Gu: None. **F. Erol:** None. **S. Tonomura:** None.

Poster

219. Somatosensation: Pain Mechanisms

Location: Hall A

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Topic: D.03. Somatosensation – Pain

Support: AR047410

Title: Characterization of NeuN, as a tool, within a free open source method of image cytometry in rat dorsal root ganglia for measurement of size and CGRP sub-populations with fluorescent immunohistochemistry during naïve and adjuvant induced arthritis (AIA) conditions

Authors: *M. B. ANDERSON, K. E. MILLER;
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Abstract: The dorsal root ganglion (DRG) contain primary afferent neurons located along the spinal column of vertebrates, projecting sensory nerves into peripheral epithelial tissues. A common method of measuring biomolecules within DRG neurons is fluorescent immunohistochemistry (IHC). Optimally this process requires manual hand tracing of neuronal boundaries, which is laborious, error-prone and can require several weeks to collect the appropriate sample size with a mouse or pen-input display monitor. The goal of the current report was to identify and characterize a reliable neuronal cytoplasmic reporter, exclusive to the DRG neuronal soma, in a semi-automated algorithm-based approach of Image Cytometry in Rat Dorsal Root Ganglia (IC-DRGs). The foundation inlying IC-DRGs processing relies upon strict requirements of size, shape and pixel-intensity criteria to eliminate error and provides

redundancy for multiple opportunities of accurate identification. Critical to this strategy, a reliable neuron-specific, non-axoplasmic, cytoplasmic fluorescent reporter was required. IC-DRGs script version 1.41 contains algorithms designed to exploit the presence of a reliable neuronal cytoplasmic reporter. These series of processes automatically assign demarcations of rat DRG neuronal cytoplasmic and nuclear membrane boundaries. Neuron-specific nuclear protein (NeuN) was identified as a consistent neuronal cytoplasm reporter in rat DRG neurons and thoroughly evaluated and qualified as a tool for use within the IC-DRGs script. 4′,6-diamidino-2-phenylindole, DAPI, a commonly used fluorescent stain which binds to DNA, was chosen as a pan-neuronal nuclear reporter. The resulting output images consist of binary neuronal nuclear and cytoplasmic masks of DRG neurons; which are then processed in CellProfiler for measurement of CGRP. Based on sixteen images per group for NeuN-IR and DAPI, IC-DRGS identified 1,136 neurons with 97.4% lenient and 94.2% conservative accuracies. We successfully show a novel approach of automated neuronal cytoplasmic and nuclear demarcations, reliable in naïve and AIA conditions, for measurement of DRG neurons using a robust FIJI script, overcoming morphological and IHC artifacts native to imaging frozen tissue sections processed with IHC.

Disclosures: M.B. Anderson: None. **K.E. Miller:** None.

Poster

219. Somatosensation: Pain Mechanisms

Location: Hall A

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Program #/Poster #: 219.13/H46

Topic: D.03. Somatosensation – Pain

Title: Optogenetic activation of primary afferents to identify postsynaptic targets in the dorsal horn

Authors: *P. J. FLYNN[1], D. M. DUBREUIL[2], B. WAINGER[2];
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Abstract: The dorsal horn of the spinal cord contains a complex network of neurons and serves as a gateway by which primary somatosensory information enters the central nervous system. Significant evidence suggests that chronic, and particularly neuropathic, pain states alter the structure and function of neural circuitry in this region, but the functional connectivity between sensory neuron subtypes and their secondary neuronal targets has not been resolved. The goal of this project is to define the dorsal horn synaptic targets of genetically-identified primary afferent subpopulations. To achieve this, we utilize optogenetic methods to non-invasively activate subpopulations of primary afferent fibers in vivo. To identify activated neurons in the dorsal spinal cord, we use immunohistochemistry to assess expression of the immediate early gene c-Fos. We demonstrate the feasibility of our approach by quantifying c-Fos-expressing cells in

spinal dorsal horn following stimulation of the plantar hindpaw of Trpv1/ChR2-EYFP mice. Activation of TrpV1-expressing nociceptors innervating the plantar hindpaw with blue light induces c-Fos expression primarily in the medial aspect of layers I and II. Furthermore, we assess the pattern of c-Fos induction by activating parvalbumin-expressing proprioceptors, which terminate in the deep dorsal horn layers III-V, and VGLUT3-expressing low-threshold mechanoreceptors, which terminate in layers I and II. Because pathological pain states may alter synaptic connections, we also investigate how neuropathic and inflammatory pain alter the synaptic connectivity of each sensory neuron subpopulation. These results will help elucidate the cellular activation patterns that underlie pathological pain, particularly how inflammatory and neuropathic pain models alter distinct labeled line activation. Analysis of these patterns may reveal particular neuronal targets that can then be interrogated further on molecular and mechanistic levels. A clearer characterization of the cell types that receive primary afferent information will create more opportunities for developing targeted therapies of treatment-resistant pain.

Disclosures: **P.J. Flynn:** None. **D.M. Dubreuil:** None. **B. Wainger:** None.

Poster

219. Somatosensation: Pain Mechanisms

Location: Hall A

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Program #/Poster #: 219.14/I1

Topic: D.03. Somatosensation – Pain

Support: NIH Grant U18 EB021716
NIH Grant R34 NS111654

Title: Targeting the nociceptive system with AAV9 and AAV2retro viral vectors

Authors: ***M. S. RIEDL**[1], A. G. J. SKORPUT[1], R. GORE[1], E. MARRON[2], K. KITTO[1], C. A. FAIRBANKS[3], L. VULCHANOVA[1];
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Abstract: Adeno-associated viruses (AAV) are widely used for neuronal transduction. AAV serotypes vary in their cell tropism and transduction efficiency via different routes of administration. In this study, we compared the biodistribution of AA9 and AAV2retro vectors delivered by routes of administration that target different components of the nociceptive system. AAV9 and AAV2retro vectors were designed to express a fusion protein of Cre-recombinase and GFP under the control of the human synapsin promoter. The viruses were delivered intrathecally to target primary afferent and spinal neurons, in the lateral parabrachial nucleus (LPbN) to target spinal dorsal horn projection neurons, and in the colon wall to target the peripheral processes of

primary afferent neurons. The biodistribution of the vectors was compared in ICR mice and/or in transgenic mice that express tdTomato in a Cre-dependent manner (Ai14 mice). Injections of the vectors in the LPbN of ICR mice revealed distinct transgene distribution based on GFP labeling visualized by immunohistochemistry. AAV9-driven GFP-Cre expression was generally restricted to regions of the midbrain in proximity to the injection site. In contrast, AAV2retro-driven GFP-Cre was also observed in rostral and caudal regions with known projections to the midbrain. This distinct pattern of distribution is consistent with the enhanced presynaptic access of AAV2retro. In the spinal cord of AAV2 injected mice, GFP-positive spinoparabrachial neurons were seen in lamina I and to a lower extent in the lateral spinal nucleus and deeper laminae. Labeled cells were seen in these regions of AAV9 injected mice but the number was approximately 25% that seen in AAV2retro injected mice. Intrathecal delivery of the vectors in Ai14 mice indicated that, based on the expression of tdTomato, AAV9 was more efficient in transducing spinal neurons whereas AAV2retro preferentially transduced primary afferent neurons. Assessment of transduction based on the virally driven expression of GFP-Cre and the Cre-dependent expression of tdTomato was vastly different, demonstrating widespread transgene expression that is below the detection limit of the virally-driven reporter. The ability of AAV9 and AAV2retro to access the peripheral processes of primary afferent neurons was evaluated by intracolonic delivery of the viruses in Ai14 mice. tdTomato expression was observed in 200-600 colon-innervating L6 dorsal root ganglion neurons in AAV9-treated mice, whereas fewer than 20 neurons expressed the reporter in AAV2retro-treated mice. This study demonstrates that AAV9 and AAV2retro can be employed to access different components of the nociceptive system.

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Poster

219. Somatosensation: Pain Mechanisms

Location: Hall A

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Program #/Poster #: 219.15/I2

Topic: D.03. Somatosensation – Pain

Support: THEA
Labex Lifesenses

Title: Up regulation of nociceptors and their role in the ocular pain physiopathology in a model of dry eye

Authors: ***D. FAKIH**[1], C. BAUDOUIN[2], A. REAUX LE GOAZIGO[3], S. MELIK PARSADANIANTZ[4];
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Saint-Quentin-en-Yvelines Univ., Paris, France; [3]UMR S 968 Inserm/Cnrs 7210, Inst. de la Vision, PARIS, France; [4]UMR S 968 Inserm/Cnrs 7210, Inst. de la Vision, Paris, France

Abstract: Dry eye disease (DED) is a multifactorial disease wherein the eyes react to trivial stimuli with abnormal sensations, such as dryness, blurring, presence of foreign body, discomfort, irritation, and pain. Despite the high prevalence of DED, the underpinning mechanisms of this ocular surface disease are not fully understood. The aim of this study was to evaluate nociceptors and their role in the DED physiopathology. DED was obtained by a unilateral excision of the extraorbital lachrymal gland (ELG) and Harderian gland (HG) in adult male mice. For sham animals, incisions were done without touching glands. Experiments were performed 21 days post excision. Behavioral studies were performed *in vivo* using von Frey filaments and capsaicine instillations. TRP expressions in the trigeminal ganglion (TG) were obtained using multiplex fluorescent in-situ hybridization (Rnascope). Corneal nerve fiber activity was evaluated by an electrophysiological approach in an *ex vivo* eye preparation by recording the multi-unit extracellular spontaneous activity of the entire ciliary nerve with a suction electrode. Besides of the ongoing activity, cold (20℃), heat (40℃), acid (pH 5 and 6) and CO_2 stimulations were done also. DED animals showed *in vivo* a corneal mechanical and chemical hypersensitivity compared to sham. Transient receptor potential (TRP): vanilloid 1 and ankyrin 1 (polymodal nociceptors) and melastatin 8 (cold nociceptor), piezo type mechanosensitive ion channel component 2 and acid-sensing ion channels (ASICs) 1 and 3 expressions were upregulated in the TG of DED animals. A significant increase of the spontaneous ciliary nerve fiber activity observed in DED animals. Besides, dryness-induced changed peripheral nociceptors responses. Cold, heat, and acid stimulations increased ongoing activity in both groups but cold and polymodal nociceptors were more activated in DED animals compared to sham. In conclusion, these results suggest that dryness caused upregulation of nociceptor expressions in the peripheral system. These peripheral upregulations are positively correlated with the nocifensif behaviour observed in DED animals and the increase of cold and polymodal activity under chemical irritation and temperature change in the cornea. Strategies to use TRP antagonist on cornea may prove beneficial as adjunct therapies in managing ocular pain in DED.

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Poster

219. Somatosensation: Pain Mechanisms

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Program #/Poster #: 219.16/I3

Topic: D.03. Somatosensation – Pain

Support: German DFG (SA-2126/2-1)

Title: Reactive dicarbonyls from the diabetic metabolism and classical inflammatory mediators work in concert to activate cutaneous nociceptors

Authors: A. K. BECKER[1], T. FLEMING[2], *P. W. REEH[1], S. K. SAUER[1];
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Abstract: Diabetes mellitus causes hyperexcitability of peripheral nociceptors and pain and hyperalgesia in about one third of diabetes patients. Certainly, a plurality of mechanisms is responsible for these neuronal disturbances. Reactive metabolites arising from the diabetic metabolism may play a considerable role. It has been shown that methylglyoxal (MG) activates cutaneous nociceptors directly through TRPA1 receptors and causes pain-related behavior when applied systemically in mice. Here we investigate effects of three classical dicarbonlys (MG, glyoxal, 3-deoxyglucosone (3-DG)) on primary sensory neurons. To date an interaction of diabetic and inflammatory tissue conditions has not been considered. We therefore explored the synergism with mediators that are typically present in inflamed tissues and conditions of tissue acidosis both likely to occur under diabetic conditions.
Recordings from a cutaneous nerve of healthy C57Bl/6J control mice showed that the classical polymodal nociceptors respond to glyoxal (10mM) superfusion of their receptive fields with an ongoing low frequency discharge pattern. A small percentage of the low threshold, rapidly adapting A-delta fibers was activated by MG (10mM) and, in contrast to C fibers, exhibited a vivid and long lasting discharge. Regarding sensory properties in C fibers, a clear tendency of threshold changes could not be detected after ten minutes of superfusion of the receptive fields whereas 43% of the responding A-delta fibers showed mechanical desensitization.
Measuring stimulated CGRP release we found that MG, glyoxal and 3-DG activate peptidergic nociceptors in a concentration-, TRPA1- and calcium- dependent manner. BK (10µM) alone caused a significant and reversible increase of CGRP release into the eluate, whereas the PGE_2 (100µM)-induced CGRP release was weak and sustained with poor reversibility. When BK (10µM) was combined with a threshold concentration of MG (1mM) a strong and supra-additive effect could be observed, whereas the combination of PGE_2 and MG caused only an additive effect. We also combined acidic stimuli (at pH 6.2 and 5.2) with MG (1mM) and found the robust low pH-induced release clearly reduced by concomitant MG application.
Reactive dicarbonyls activate distinct sensory nerve fibers in the skin and release CGRP. In addition, a differential synergism with inflammatory tissue conditions is conceivable and requires further investigation especially under diabetic conditions.
The *ex vivo* animal research plan was approved by the Government of Lower Franconia in Würzburg.

Disclosures: **A.K. Becker:** None. **T. Fleming:** None. **P.W. Reeh:** None. **S.K. Sauer:** None.

Poster

219. Somatosensation: Pain Mechanisms

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Topic: D.03. Somatosensation – Pain

Support: CIHR Grant MOP-130471
NSERC Discovery Grant 436091
CIHR Grant PJT-153183

Title: Lionfish venom elicits pain predominantly through the activation of non-peptidergic nociceptors

Authors: *S. MOUCHBAHANI-CONSTANCE[1,2], L. S. LESPERANCE[3,4], H. PETITJEAN[1,2], A. DAVIDOVA[1], S. A. PRESCOTT[3,4], R. SHARIF-NAEINI[1,2];
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Abstract: The lionfish (*Pterois volitans*) is a venomous invasive species found in the Caribbean and Northwestern Atlantic. It poses a growing health problem because of the increase in frequency of painful stings, for which no treatment or antidote exists, and the long-term disability caused by the pain. Understanding the venom's algogenic properties can help identify better treatments for these envenomations. In this study, we provide the first characterization of the pain and inflammation caused by lionfish venom and examine the mechanisms through which it causes pain using a combination of *in vivo* and *in vitro* approaches including behavioural, physiological, calcium imaging and electrophysiological testing. Intraplantar injections of the venom produce a significant increase in pain behaviour, as well as a marked increase in mechanical sensitivity for up to 24 hours after injection. The algogenic substance(s) are heat-labile peptides that (1) cause neurogenic inflammation at the site of injection, as well as (2) induction of Fos and (3) microglia activation in the superficial layers of the dorsal horn. Finally, calcium imaging and electrophysiology experiments show that the venom acts predominantly on nonpeptidergic, TRPV1-negative, nociceptors, a subset of neurons implicated in sensing mechanical pain. These data provide the first characterization of the pain and inflammation caused by lionfish venom, as well as the first insight into its possible cellular mechanism of action.

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Poster

219. Somatosensation: Pain Mechanisms

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Topic: D.03. Somatosensation – Pain

Support: P20GM103643

Title: Assessing role of non-peptidergic MrgD expressing fibers in cancer-induced bone pain

Authors: K. GAUDREAU[1], ***J. J. HAVELIN**[2], T. E. KING[1];
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Abstract: Cancer-induced bone pain is reported to be one of the most detrimental aspects of the disease often broadly categorized into two separate phenomena. Patients experience ongoing pain, a dull achy persistent background pain that worsens as disease progresses and is currently treated with around the clock mu opioid receptor (MOR) agonists. In addition, patients often report transient episodes of severe pain that is sometimes spontaneous but often triggered by movement and that "breaks through" the around the clock medication. Breakthrough pain is treated with additional rapid onset MOR agonists that are limited by dose- limiting side effects and often miss an effective window of treatment for the patient. We examined the hypothesis that ongoing pain and breakthrough pain are initiated by different populations of sensory afferents. Data presented will expand upon our previous observations in a rat model of cancer-induced bone pain that IB4-binding fibers play a critical role in transducing breakthrough pain whereas TRPV1 expressing fibers do not. We are characterizing cancer-induced ongoing and breakthrough bone pain in a mouse model. Lewis lung carcinoma (LLC) cells are injected and sealed into the femur of C57bl/6 mice. Nav1.8-Cre and MrgD-ERT2-Cre mice have been crossed with tdTomato mice to examine potential pathological changes in bone innervation in tumor bearing mice. In agreement with previous studies, we observe pathological sprouting within the bone and periosteum of the tumor bearing bones. We are also examining overlay of markers for neuronal damage within DRG innervating the femur in both lines. In addition, we are using pharmacological and optogenetic techniques to assess subpopulations of sensory fibers in both ongoing and breakthrough pain. Pharmacological characterization of cancer-induced ongoing pain demonstrates that the peptidergic MOR agonist DAMGO induces CPP whereas the peptidergic DOR agonist Deltorphin does not. In contrast, preliminary data indicate that Deltorphin blocks movement-induced breakthrough pain. We have also demonstrated that silencing Nav1.8 expressing fibers blocks tumor-induced ongoing pain whereas MrgD expressing fibers do not. We are exploring the ability of optogenetic silencing of Nav1.8 compared to MrgD expressing fibers in mediating breakthrough pain in the mouse. This research is supported by a COBRE award (P20GM103643).

Disclosures: **J.J. Havelin:** None. **T.E. King:** None. **K. Gaudreau:** None.

Poster

219. Somatosensation: Pain Mechanisms

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Topic: D.03. Somatosensation – Pain

Support: NIH OT2OD023854

Title: Development of a TRPA1 reporter mouse model

Authors: S.-H. KIM[1], P. K. BAHIA[2], J. HARSANYIOVA[3], M. KOLLARIK[3], ***T. TAYLOR-CLARK**[3];
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Abstract: Transient Receptor Potential Ankyrin 1 (TRPA1) is an ion channel which is activated by environmental irritants (e.g. formaldehyde and acrolein) and pungent stimuli such as mustard oil, garlic and cinnamaldehyde. TRPA1 is known to be expressed in a subset of nociceptive sensory afferent and thus, playing an important role in defensive reflexes. However, TRPA1 has also been implicated in fibroblast, epithelial cell and smooth muscle cell function, thus it is not entirely clear the extent to which TRPA1 function is restricted to sensory neurobiology. In the current study, we used the Flp-FRT (flipase-flipase recognition target) system to generate a TRPA1 reporter mouse model. We generated a TRPA1-Flp knockin stain which incorporated Flp_o after the last endogenous TRPA1 exon. Expression of Flp_o was linked to the expression of endogenous TRPA1 via a 2A sequence. The TRPA1-Flp was crossed with a tdTomato reporter strain carrying *frt*-flanked STOP condon (RC::FLTG) to generate a mouse model (Flp::RC) that expresses red fluorescent protein tdTomato in TRPA1 expressing cells. Vagal sensory ganglia were collected from those animals and used for characterization. First, the vagal ganglia were cryosectioned and immunostained to determine nociceptive subtypes using anti-TRPV1 antibody. Second, primary neurons were isolated from the vagal ganglia and used to monitor calcium influx changes after stimulation with allyl isothiocyanate (AITC), the TRPA1 agonist. tdTomato was expressed in a subset of vagal ganglion neurons, but not in other cell types within the ganglion. The majority of the red fluorescent cells were TRPV1 positive cells and approximately 30% of TRPV1 positive cells co-expressed TRPA1. In our functional studies, a total of 58 dissociated vagal neurons were assayed and 17 cells expressed tdTomato. Among the tdTomato expressing cells, 11 cells responded to 100 μM AITC. Additionally, 9 cells responded to AITC from 41 of non-tdtomato expressing cells. Overall, our data suggests that the transgenic mouse model generated using Flp-FRT system successfully labeled TRPA1 cells. Our

preliminary findings suggest that the TRPA1 Flp::RC strain can be utilized in investigations involving TRPA1 ion channel physiology.

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Poster

219. Somatosensation: Pain Mechanisms

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Support: NIH Grant P20GM103643
NIH GM RO1102346

Title: A novel mechanism for regulation of mitochondrial function by Epac2 contributes to acute hyperalgesia evoked by prostaglandin E2

Authors: D. J. GOODE, *D. C. MOLLIVER;
Biomed. Sci., Univ. of New England, Biddeford, ME

Abstract: Prostaglandin E2 (PGE2) is a well-known inflammatory mediator that promotes inflammation and behavioral hypersensitivity in response to tissue damage. PGE2 signaling through Gs-coupled EP4 receptors induces acute nociceptor sensitization and thermal and mechanical hyperalgesia mediated by protein kinase A. Conversely, the alternate cAMP effector Epac has been implicated in some forms of chronic, but not acute, hyperalgesia. Here, we demonstrate that Epac2 contributes to acute heat hyperalgesia through a mechanism involving the positive regulation of mitochondrial function. PGE2 administered to dissociated dorsal root ganglion (DRG) neurons *in vitro*, or injected into the mouse hindpaw *in vivo*, leads to a rapid activation of PKC that is blocked by the selective Epac2 inhibitor HJC0350. Furthermore, hindpaw injection of HJC0350 attenuated acute PGE2-evoked heat hyperalgesia. Previously, we used phospho-protein profiling-mass spectrometry to identify pyruvate dehydrogenase (Pdha1) as a major downstream target of Epac-PKC signaling in DRG neurons from adult male and female mice. Using Seahorse metabolic flux analysis, we found that PGE2 enhanced maximal respiration and ATP production. PGE2 increased production of reactive oxygen species (ROS), measured by flow cytometry with the ROS indicator MitoPY1. This effect was blocked by HJC0350. These findings identify a novel mechanism for positive regulation of mitochondrial function by Gs-coupled receptors signaling through Epac2. Canonical regulation of Pdha1 occurs through inhibition by pyruvate dehydrogenase kinases (PDK), which reduces mitochondrial ATP production, buffering of intracellular calcium and ROS. Inhibition of PDKs with dichloroacetic acid enhanced the PGE2-induced increase in mitochondrial respiration in dissociated DRG

neurons *in vitro,* and prolonged PGE2-evoked heat hyperalgesia from 2 hours to at least 24 hours *in vivo* (n=5/condition/sex). HJC0350 suppressed the enhancement of respiration by PGE2 *in vitro*. Systemic administration of either HJC0350, or a mild mitochondrial membrane potential uncoupler (2,4-dinitrophenol), attenuated PGE2-induced heat hyperalgesia (n=5/condition/sex). Scavenging of ROS with systemic mitoquinol (an antioxidant targeted to mitochondria) failed to prevent PGE2-evoked thermal hyperalgesia. Experimenters were blinded to drug treatments. Our results indicate that Gs-coupled Epac2 signaling contributes to acute hyperalgesia through the positive regulation of mitochondrial function in both male and female mice.

Disclosures: D.C. Molliver: None. **D.J. Goode:** None.

Poster

219. Somatosensation: Pain Mechanisms

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ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: D.03. Somatosensation – Pain

Support: NIH grant NS065926
NIH grant NS102161

Title: Correlational gene modules in human PNS tissues help identify key regulatory and signalling pathways related to nociception and neuro-immune interactions

Authors: *P. R. RAY[1], A. WANGZHOU[3], V. JEEVAKUMAR[4], T. J. PRICE[2];
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Abstract: Aims and Rationale: Recent interest in high throughput mRNA profiling of the human PNS has led to a comprehensive picture of the transcriptional landscape of the human DRG and tibial nerve [Sapio et al, Exptal Neurosci 2016; Ray et al, PAIN 2018]. Discriminative gene sets that are hallmarks of neuropathic pain cohorts have also been identified [North et al, Brain 2019]. However, a diversity of processes underlie human neuropathies, which are often the result of a diverse interaction of genetics, environment, lifestyle factors and accompanying pathologies, confounding analysis. Additionally, incomplete or incorrect demographic and clinical records can further complicate cohort-based analysis. We performed unsupervised analysis that identified gene co-expression modules in the human DRG and tibial nerve. Methods: We based our human DRG analysis on RNA-seq datasets from the UT Dallas hDRG dataset (>40 samples), and the tibial nerve analysis on the GTEx consortium data (>500 samples). Pearson Correlation of suitably standardized expression values were calculated for genes with variable expression.

Correlation based networks were then analyzed to identify densely connected components to generate co-expression modules in both the DRG and tibial nerve. Finally, we performed enrichment analysis of sample properties to identify enriched demographic and clinical variables for each co-expression module. Results: Our results identify co-expression modules that are linked to expression changes in transcriptional regulators like members of the FOS/JUN family. Coexpression modules involving nociceptive molecules like BDNF also identified potentially co-regulated genes like GAL, CCL5, and THY1. Enrichment analysis of the modules in DRG show sexually dimorphic components, while for the tibial nerve, we were able to identify co-expression modules enriched in diabetic patients. Technical variables affecting sample quality (like the proportion of neurons that are excised per sample) can be identified using specific diagnostic co-expression modules. Conclusion: Our approach identifies co-expression modules that capture key aspects of biological and technical variability in human DRG and tibial nerve samples, and serve as a roadmap for gene expression analysis in clinical settings.

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Poster

219. Somatosensation: Pain Mechanisms

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Topic: D.03. Somatosensation – Pain

Support: NIH/NIAMS Grant AR068012

Title: Effects of cannabinoid agonist on human sensory neurons

Authors: *S. DAVIDSON[1], A. N. REKER[2], Z. K. FORD[3], S. CHEN[1];
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Abstract: Cannabinoids have been suggested as a potential alternative approach for pain relief. The goal of the present study was to determine whether activation of cannabinoid receptors in human sensory neurons could suppress nociceptive responses or hyperactivity. Lumbar level human dorsal root ganglia were recovered from cadaveric organ donors at the University of Cincinnati Medical Center, dissociated and cultured, or fixed and frozen for histology. Immunohistochemistry experiments demonstrate that the CB1 receptor is expressed by approximately 85% of human sensory neurons. Using Fura-2AM calcium imaging of viable human sensory neurons, responses to the peripherally active cannabinoid agonist CB-13, a potent CB1/CB2 agonist, in naïve neurons were examined. Responses to capsaicin (250 nM) in presence and absence of the inflammatory mediator PGE2 (1 µM) were examined after pre-treatment with 1 µM CB-13, or with CB-13 bath applied in between capsaicin administrations.

Our results show that CB-13 produced no calcium influx in sensory neurons on its own, and that responses to capsaicin in naïve neurons as well as PGE2-exposed neurons were surprisingly enhanced by CB-13. Patch clamp recordings of human sensory neurons were performed to test changes in membrane properties by cannabinoid receptor activation by CB-13. In these experiments, CB-13 suppressed action potential discharge relative to PGE2 alone supporting the concept that peripherally acting cannabinoid receptors may have the capacity to reduce sensory neuron nociceptive responses.

Disclosures: **S. Davidson:** None. **A.N. Reker:** None. **Z.K. Ford:** None. **S. Chen:** None.

Poster

219. Somatosensation: Pain Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 219.23/I10

Topic: D.03. Somatosensation – Pain

Support: NIH Grant RO1 NS091759
NIH Grant T32 GM120011
NIH Grant RO1 NS111521
NIH Grant F31 GM133203

Title: Mechanisms that drive enhancement of depolarizing spontaneous fluctuations of membrane potential and associated ongoing activity in nociceptors

Authors: *E. R. LOPEZ[1], M. A. ODEM[2], A. GARZA-CARBAJAL[1], A. G. BAVENCOFFE[1], C. W. DESSAUER[1], E. T. WALTERS[1];
[1]Integrative Biol. and Pharmacol., [2]Microbiology and Mol. Genet., The Univ. of Texas Hlth. Sci. Ctr. At Houston, Houston, TX

Abstract: Hyperexcitability in nociceptors is an important driver of ongoing pain. Nociceptors in a hyperexcitable state often exhibit ongoing activity (OA), the continuing discharge of action potentials. Two well-recognized electrophysiological alterations that promote OA are prolonged depolarization of resting membrane potential and reduction of action potential threshold. A recent study identified increased amplitude of depolarizing spontaneous fluctuations (DSFs) of membrane potential as a third important contributor to OA in nociceptors. We recently demonstrated that OA can be potentiated acutely by the inflammatory mediator serotonin, in part by enhancing DSFs under moderately depolarized conditions. Thus, acute treatment with serotonin combined with artificial depolarization provides a model for detailed mechanistic studies of DSFs and associated OA in DRG neurons from naïve rats. The objective of this study is to identify the cell signaling mechanisms critical for enhancement of OA in the serotonin-induced model of nociceptor hyperexcitability. Based on previous studies and preliminary

observations, I hypothesize that peripheral serotonin modulates specific conductances via cAMP signaling downstream of Gs-coupled receptor activation to generate large DSFs that promote OA in nociceptors. Dose-response relationships indicate that relatively low concentrations of serotonin, 10-300 nM, are most effective at promoting nociceptor hyperexcitability. In whole-cell patch recordings, an inhibitor of protein kinase A (PKA) strongly reduced serotonin enhancement of DSFs and potentiation of OA. An inhibitor of exchange factor directly activated by cAMP1 (EPAC1) prevented serotonin reduction of action potential threshold but did not significantly attenuate serotonin-induced OA. Furthermore, intracellular application of a cAMP analog potentiates OA. These results suggest that OA potentiated acutely by relatively low concentrations of serotonin involves cooperative cAMP signaling through PKA and EPAC and demonstrate that increased intracellular cAMP is sufficient to potentiate ongoing activity. Further investigation into the receptors, intracellular effectors, and ion channels that mediate OA in nociceptors may lead to novel therapeutic strategies for alleviating ongoing pain.

Disclosures: **E.R. Lopez:** None. **M.A. Odem:** None. **A. Garza-Carbajal:** None. **A.G. Bavencoffe:** None. **C.W. Dessauer:** None. **E.T. Walters:** None.

Poster

219. Somatosensation: Pain Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 219.24/I11

Topic: D.03. Somatosensation – Pain

Support: Mission Connect, a program of TIRR Foundation, Grant 017-107

Title: Macrophage migration inhibitory factor (MIF) may contribute to pain after spinal cord injury by promoting ongoing activity in primary nociceptors

Authors: *A. G. BAVENCOFFE[1], A. GARZA CARBAJAL[1], E. A. SPENCE[1], O. E. BLOOM[2], C. W. DESSAUER[1], E. T. WALTERS[1];
[1]Integrative Biol. and Pharmacol., McGovern Med. Sch. at UTHealth, Houston, TX; [2]The Feinstein Inst. for Med. Res., Manhasset, NY

Abstract: Chronic neuropathic pain afflicts more than half of patients with spinal cord injury (SCI) and impairs quality of life. The mechanisms are poorly understood and treatments remain inadequate. Critical mechanisms promoting chronic pain are located within nociceptors, which exhibit hyperactivity, including spontaneous and ongoing activity (OA), that contributes to ongoing pain after SCI (Qing et al., J Neurosci. 34: 10765, 2014). We showed that cAMP-dependent pathways are required to maintain SCI-induced OA (Bavencoffe, Li et al., J Neurosci. 36: 1660, 2016). However, these pathways are nearly ubiquitous and unsuitable for selective therapeutic targeting. Nociceptors after SCI become hypersensitive to chemical signals linked to

inflammation and injury, including TRPV1 agonists and serotonin. Due to the lack of an effective vascular permeability barrier in DRGs, and their intrathecal location, nociceptor somata are exposed to chemical signals in both blood and cerebrospinal fluid. In humans, SCI causes acute and chronic elevation in circulating levels of cytokine macrophage migration inhibitory factor (MIF) (Stein et al., Arch Phys Med Rehabil, 94:1498, 2013), and MIF was identified as a key factor in other models of inflammatory and neuropathic pain.
We previously identified two types of nociceptor *in vitro* based on differences in accommodation to prolonged depolarizing pulses: rapid accommodating (RA) and nonaccommodating (NA), with only the latter capable of exhibiting OA (Odem et al., Pain 159:2347, 2018). We find that MIF only stimulates NA neurons. Moreover, MIF, at levels reported in SCI patients' plasma (1 ng/ml), potently switches nociceptors into a hyperexcitable OA state comparable to the one observed after SCI, with 72% of NA neurons exhibiting OA (compared to 9% in control). MIF dose-dependently enhances all three general electrophysiological properties that can promote OA: depolarization of resting membrane potential, hyperpolarization of action potential threshold, and enhancement of the amplitude and incidence of depolarizing spontaneous fluctuations. Nociceptors isolated from SCI rats displayed a higher sensitivity to MIF compared to sham controls. Behavioral studies using conditioned place preference and avoidance tests suggest that MIF contributes to spontaneous and evoked pain in rats. These findings suggest that therapeutic inhibition of MIF after SCI could reduce pain by reducing nociceptor hyperactivity.

Disclosures: **A.G. Bavencoffe:** None. **A. Garza Carbajal:** None. **E.A. Spence:** None. **O.E. Bloom:** None. **C.W. Dessauer:** None. **E.T. Walters:** None.

Poster

219. Somatosensation: Pain Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 219.25/I12

Topic: D.03. Somatosensation – Pain

Support: Australian NHMRC

Title: Identifying sensory nerve endings that innervate the bone marrow and periosteum using anterograde tracing

Authors: J. THAI[1], M. KYLOH[2], L. TRAVIS[2], N. SPENCER[2], ***J. J. IVANUSIC**[1];
[1]Anat. and Neurosci., Univ. of Melbourne, Melbourne, Australia; [2]Flinders Univ., Adelaide, Australia

Abstract: Whilst sensory and sympathetic neurons are known to innervate bone, previous studies have found it difficult to unequivocally identify only those that are of sensory origin. In this study, we have used anterograde tracing to selectively label spinal afferent (sensory) nerve

endings that innervate the periosteum and bone marrow. 9 males and 4 female C57BL/6 mice, aged 11-17 weeks, were used in this study. Mice were anesthetized and dextran-biotin (anterograde tracer; 50-100nl, 10-20% in saline) was injected unilaterally into L3-L5 dorsal root ganglia. Mice were given a 9-day recovery period to allow sufficient time for anterograde transport of the tracer to nerve terminal endings in bone. The periosteum (wholemount) and underlying bone were collected from both the femur and tibia. These tissues were processed to reveal anterograde labelling, and immuno-labelled with antibodies directed against protein gene product (PGP9.5; pan-neuronal marker), tyrosine hydroxylase (TH; sympathetic neuron marker) or calcitonin gene-related protein (CGRP; peptidergic nociceptor marker) to classify nerve fibers on the basis of their neurochemical profile. Anterograde labelled nerve endings were dispersed throughout the periosteum and marrow cavity, and could be identified in close apposition to blood vessels and at sites distant from them. The anterograde labelled nerve endings did not express TH, and lacked the circumferential arrangement around blood vessels characteristic of sympathetic innervation. Many of the anterograde labelled nerve endings expressed CGRP but some did not, suggesting the presence of both peptidergic and non-peptidergic nociceptor phenotypes. Further experiments are required to confirm this distinction. This approach to selective labelling of sensory nerve terminal endings helps to better identify how different sub-populations of sensory neurons, and their peripheral nerve terminal endings, interact with bone.

Disclosures: **J.J. Ivanusic:** None. **J. Thai:** None. **N. Spencer:** None. **M. Kyloh:** None. **L. Travis:** None.

Poster

219. Somatosensation: Pain Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 219.26/I13

Topic: D.03. Somatosensation – Pain

Support: NIH R01 GM124055
NIH R01 DK116624

Title: Evaluation of incision-induced sensitization of peripheral sensory afferents in muscle using *in vivo* GCaMP3 imaging

Authors: D. SUGIYAMA[1], A. L. KEYES[2], Y. M. USACHEV[3], T. J. BRENNAN[4], *S. KANG[5]; [1]Dept. of Anesthesia, [2]Univ. of Iowa, Iowa City, IA; [3]Dept Pharmacol, Univ. of Iowa Dept. of Pharmacol., Iowa City, IA; [4]Dept. of Anesthesia, Univ. of Iowa Col. of Med., Iowa City, IA; [5]Universit of Iowa Hosp. and Clinics, Iowa City, IA

Abstract: We have previously shown that endogenous TRPA1 agonists, such as hydrogen peroxide and 4-hydroxynonenal (4-HNE), are increased in the wound environment after incision

(1). Moreover, the Increase in Ca^{2+} influx evoked by TRPA1 agonists was greater in the dorsal root ganglion (DRG) neurons innervating deep tissue, compared with skin DRG neurons after incision (2). In this study, we performed the *in vivo* GCaMP3 imaging of peripheral sensory afferents in deep tissue.

Male and female Pirt-GCaMP3 mice (20-30 g) were used. These mice express GCaMP3, a genetically encoded Ca^{2+} indicator, in most nociceptive DRG neurons under the control of Pirt promoter (3,4). One day after hind paw incision, *in vivo* Ca^{2+} imaging of hind paw muscle was performed under anesthesia. All experiments were performed at room temperature (~25 ℃). The imaging mount was set on the exposed flexor digitorum brevis muscle. Two-photon Ca^{2+} imaging was performed with Olympus multiphoton confocal microscope FVMPE-RS equipped with Mai Tai DeepSee laser (690-1040 nm), and 20x objective and a Z-deck motorized stage. GFP signals were measured to see Ca^{2+} transients using green emitted light. Data were expressed as $\Delta F/F_0 = (F-F_0)/ F_0$ as a function of time (F is the current fluorescence intensity and F_0 is the fluorescence intensity in the baseline period). Baseline Ca^{2+} transients and response to 4-HNE (microinjected to the imaging area; 100μM in 50 μl) were compared between the incision *vs*. control (un-incised) group.

The baseline level of Ca^{2+} transients for 3 minutes in the incision group (1.09 ± 0.22, mean ± SD) was greater than the control group (0.88 ± 0.15; p = 0.0417). The time-averaged area under the curve (AUC) of the baseline Ca^{2+} transients in the incision group (65.1 ± 13.3/min) was also greater than the control group (52.2 ± 8.7; p = 0.0369). After 4-HNE injection, AUC of Ca^{2+} transients in the incision group (71.1 ± 19.4/min) was greater than the control group (51.8 ± 6.7; p = 0.0188). On the other hand, there was no difference in the increased ratio of the Ca^{2+} transients from the baseline after 4-HNE injection between the incision (112.9 ± 16.7) and control group (101.3 ± 20.5; p = 0.2361).

In conclusion, our *in vivo* Pirt-GCaMP3 mice imaging data indicate that the spontaneous Ca^{2+} transients of the peripheral sensory nerves in muscle after incision was greater than un-incised muscle. Our results also suggested that the response to TRPA1 agonist of the peripheral afferents in deep tissue after the incision was also greater than the control group.

References

1. Sugiyama *et al*. PLoS One 12:e0170410
2. Sugiyama *et al.* Neuroscience 2018 Meeting, San Diego, CA
3. Kim *et al*. Cell 133:475-85, 2008
4. Kim *et al.* Neuron 81:873-87, 2014

Disclosures: D. Sugiyama: None. **A.L. Keyes:** None. **Y.M. Usachev:** None. **T.J. Brennan:** None. **S. Kang:** None.

Poster

219. Somatosensation: Pain Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 219.27/I14

Topic: D.03. Somatosensation – Pain

Support: MOST 107-2321-B-002-006

Title: Correlation between nerve injury and evoked neuropathic pain in chronic constriction injured mice

Authors: *H.-H. CHI, J.-C. LEE, R.-F. CHEN, C.-T. YEN;
Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Chronic constriction injury of the sciatic nerve (CCI) is a model for neuropathic pain after compression injury of the peripheral nerve. Voltage-gated sodium channel 1.8 ($Na_v1.8$) is a slow sodium channel highly expressed in small sensory fibers. It plays a crucial role in noxious mechanical and thermal pain sensations. Cumulative literature reveals that $Na_v1.8$-expressing neuron is one of the major players in the pain onset and hypersensitivity under chronic pain condition. Although it is well-known that many peripheral nerves degenerate after compression nerve injury, it is still unclear, however, how the extend of nerve changes correlates with the severity of pain. We used intra-vital two-photon microscopic methods to longitudinally observe the cutaneous fiber changes in the hind paw of a $Na_v1.8$ knockin mice line ($Na_v1.8$-cre::tdTomato) after CCI. Results showed that CCI caused primary sensory neuron injury and loss of skin nerve plexus and intraepidermal nerve fibers. The timing of the loss of skin nerve plexus and intraepidermal nerve fibers correlated with the development of mechanical hypersensitivity and thermal hyperalgesia. We found a U-shape correlation between the remaining hind paw fiber and the mechanical threshold of a von Frey hair, such that the most severe evoked hypersensitivity was observed in the intermediately injured mice. A linear correlation was found between the mechanical threshold versus the multiplication of the amount of nerve injured and the amount of the nerve remained. This implies an interaction of the injured nerves and the remaining nerves to produce the evoked mechanical allodynia.

Disclosures: H. Chi: None. **J. Lee:** None. **R. Chen:** None. **C. Yen:** None.

Poster

219. Somatosensation: Pain Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 219.28/I15

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant DK100460
NSF Grant 1727185

Title: Submucosa is the load bearing structure of distal colon and rectum

Authors: *S. SIRI[1], F. MAIER[2], S. SANTOS[2], D. PIERCE[2], B. FENG[1];
[1]Biomed. Engin., [2]Mechanical Engin., Univ. of Connecticut, Storrs, CT

Abstract: Visceral pain from distal colon and rectum (colorectum) has a unique biomechanical aspect: it is mechanical colorectal distension/stretch — not heating, pinching, or cutting the colorectum — that effectively evokes the perception of pain. Our recent study reveals significant differences in local biomechanical properties at different regions along the longitudinal colorectum. The current study focuses on the layered structure of the colorectum across the wall thickness and determines the biomechanical properties of layer-separated colorectal tissue. We harvested the distal 30 mm of the colorectum from mice and performed fine dissection to separate into inner and outer composite layers from the interstitial space under the submucosa. The inner composite consists of the mucosa and submucosa while the outer composite includes the muscular layers and serosa. We then divided each composite longitudinally into three 10-mm-long segments (colonic, intermediate, and rectal) and conducted biaxial mechanical stretch tests and opening-angle measurements for each tissue segment. In addition, we quantified the morphology and geometry of the rich collagen network in the submucosal layer by nonlinear imaging via second harmonic generation (SHG). Our results reveal signficantly higher stiffness of the inner composite than the outer composite in both axial and circumferential directions. The tissue is anisotropic at all regions of the colorectum with higher stiffness in the axial direction than in the circumferential direction, and tissue anisotropy is more pronounced in the inner composite than in the outer one. The stiffness of the inner composite in the axial direction is about twice that in the circumferential direction, consistent with the orientations of collagen fibers in the submucosa approximately ± 30 degrees to the axial direction. Strikingly, the axial stress - stretch relations are comparable across all three regions in the inner composite (colonic, intermediate and rectal) despite their significant differences in thickness, which is likely due to the comparable thickness and morphology of network of collagen fibers throughout the longitudinal directions in the submucosa. These biomechanical and morphological results strongly indicate the submucosa as the load-bearing structure of the colorecum. This, in turn,

implies nociceptive roles for the colorectal afferent endings in the submucosa that likely encode tissue-injurious mechanical distension/stretch to inform the central nervous system.

Disclosures: **S. Siri:** None. **F. Maier:** None. **S. Santos:** None. **D. Pierce:** None. **B. Feng:** None.

Poster

219. Somatosensation: Pain Mechanisms

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 219.29/I16

Topic: F.03. Neuroendocrine Processes

Support: NIH SPARC Grant 2OT2OD023861-01
Florida Veterinary Scholars Program

Title: Pancreatic sensory neurons in the DRG and nodose ganglion have different immunohistochemical/morphometric signatures for TRPA1, P2X3, NFM, and CGRP

Authors: L. E. HAIRE, T. L. REDLER, V. P. DUGAN, A. KUNDU, *R. D. JOHNSON; Physiological Sci., Univ. of Florida, Gainesville, FL

Abstract: Diabetes is a significant autoimmune disease affecting both humans and animals, and there is no current treatment that fully mitigates the negative health consequences and life style changes. Neuromodulation of pancreatic nerves (stimulation/blocking) may be useful in next-generation treatments to improve insulin secretion and quality of life for diabetic patients, but a precise map of the sensory and motor nerve pathways is required. The pancreas is richly innervated, playing an integral role in hormone secretion, blood flow, inflammation, islet function, and sensation. In the present nerve mapping study, we used DiI to retrogradely label pancreatic afferent neurons in the thoracic dorsal root ganglia (DRG) and vagal nodose ganglia (NG) in normal rats. Using immunohistochemistry, DiI-labeled cells were examined for the presence and co-localization of four markers: transient receptor potential ankyrin-1 (TRPA1), ATP receptor P2X3, myelination marker Neurofilament M (NFM), and vascular afferent marker calcitonin gene-related peptide (CGRP). Aseptic surgical exposure of the pancreas in anesthetized mature male Sprague-Dawley rats was followed by several 1µL injections of a retrograde fluorescent tracer, DiI paste, delivered through a beveled glass micropipette via pressure injector pulses. After 19-20d recovery, the animals were euthanized and transcardially perfused with PBS at pH 7.4 and 4% paraformaldehyde. Bilateral T8-T11 DRGs and NGs were dissected free, post-fixed overnight and cryoprotected in 30% sucrose solution. Serial ganglion cryosections at 14µm were thaw-mounted onto two alternating slides. Nucleated DiI-positive cells/axons were visualized via standard multi-label IHC fluorescence microscopy (Keyence) and the images digitized. Results in the DRG showed a bilateral subset of pancreatic DRG neurons

were positive for TRPA1, always co-localized with CGRP, and less than half were NFM positive. Pancreatic DRG neurons were P2X3 negative. In contrast to the DRG, approximately half of pancreatic NG neurons were positive for P2X3 and TRPA1, had significantly smaller diameters, and mostly lacked myelination marker NFM and vascular afferent marker CGRP. We conclude (i) pancreatic sensory neurons in both the DRG and NG have receptors (TRPA1) to cinnamaldehyde/allyl isothiocyanate, two dietary compounds used in the treatment of hyperglycemia, (ii) the complete co-localization of TRPA1 and CGRP in the spinal pathway, but not the vagal pathway, suggests a role in the control of pancreatic blood flow, and (iii) P2X3 labeling only in NG suggests an unperceived pancreatic nociceptive/regulatory role.

Disclosures: L.E. Haire: None. **T.L. Redler:** None. **V.P. Dugan:** None. **R.D. Johnson:** None. **A. Kundu:** None.

Poster

220. Preclinical and Clinical Studies in Peripheral Nerve Injury and Neuropathic Pain

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 220.01/I17

Topic: D.03. Somatosensation – Pain

Support: UK Medical Research Council

Title: Epigenetic profiling of sensory neurons in chronic pain models

Authors: *S. VILLA[1], F. DENK[2];
[1]King's Col. of London, London, United Kingdom; [2]King's Col. London, London, United Kingdom

Abstract: Objective and Rationale: Chronic pain conditions are an important clinical problem, affecting 20% of the population world-wide. Most available treatments have limited efficacy and serious side effects. While it is known that chronic pain conditions are significantly driven by hypersensitivity of peripheral sensory neurons, the root cause of this persistent hypersensitivity is less well understood. Here, we are investigating whether epigenetic changes, specifically enhancer remodelling, might be responsible for the long-lasting changes in peripheral gene expression and function that are known to occur in animal models of chronic pain. **Methods:** Transgenic Adv-CreERT2-Sun1GFP mice were generated to permit specific labelling of sensory neuron nuclei. Mice then underwent partial sciatic nerve ligation or sham surgery and were sacrificed seven days later. Lumbar dorsal root ganglia (DRG) were dissected and nuclei were extracted from Advillin-GFP neurons using Fluorescence-Activated Cell Sorting (FACS). Chromatin profiling was performed using ChIP-seq and an adapted CUT&RUN protocol (Henikoff, 2019) for Ultra Low inputs (uliCUT&RUN; Fazzio, 2019) with several histone marks (H3k4me1, H3k27ac) and transcriptional regulators like CTCF and Cohesin. **Results:** H3K4me1

marks were unchanged by nerve injury, indicating that basic enhancer positioning remained intact. CUT&RUN optimisation suggests that a few thousand nuclei will be sufficient to visualise epigenetic modifications in sensory neurons. **Conclusions:** We can now examine the full spectrum of enhancers and epigenetic modifications in small populations of sensory neurons. We will use this technology to analyse different enhancer histone marks, such as H3K27ac, and chromatin regulators, such as CTCF, cohesin and CBP, to study epigenetic changes in mouse models of neuropathic pain.

Disclosures: S. Villa: None. **F. Denk:** None.

Poster

220. Preclinical and Clinical Studies in Peripheral Nerve Injury and Neuropathic Pain

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 220.02/I18

Topic: D.03. Somatosensation – Pain

Support: NIH NINDS R01NS102836

Title: The role of small extracellular vesicles in chronic neuropathic pain

Authors: *Z. LIN[1], S. K. AJIT[2], R. JEAN-TOUSSAINT[2], Y. TIAN[1], A. SACAN[3]; [2]Pharmacol. & Physiol., [1]Drexel Univ. Col. of Med., Philadelphia, PA; [3]Drexel Univ. Sci. & Hlth. Systems, Philadelphia, PA

Abstract: Exosomes are 30-150 nm extracellular vesicles that can transport RNAs, proteins, and lipid mediators to recipient cells via circulation. Exosomes can be beneficial or harmful depending on their source and contents. We hypothesized exosome content would be altered following nerve injury and these alterations can provide insight into signaling mechanisms involved in neuropathic pain. To characterize exosome composition following nerve injury, small extracellular vesicles (sEVs) were purified from mouse serum four weeks after spared nerve injury (SNI) or sham surgery. Our miRNA profiling showed a distinct miRNA signature in SNI model compared to sham control. Proteomics analysis using tandem mass spectrometry detected 274 gene products. Of these, 24 were unique to SNI model. In addition to commonly expressed exosome proteins, multiple members of serpin and complement family were detected in exosomes. Neuropathic pain can induce the activation of the complement cascade and our cytokine profiling showed significant upregulation of complement component 5a (C5a) in sEVs from SNI model. Intercellular Adhesion Molecule 1 (ICAM-1), required for the leukocyte recruitment, adhesion and homing of exosomes was also upregulated in sEVs from SNI model compared to sham control. We observed a differential distribution of C5a and ICAM-1 within serum and sEVs between sham and SNI, indicating changes from local or paracrine to long distance signaling under neuropathic pain. Our studies suggest critical roles for cargo sorting of

vesicular proteins in mediating neuropathic pain. In vivo studies are ongoing to determine the functional significance of alterations in exosome composition.

Disclosures: Z. Lin: None. **S.K. Ajit:** None. **R. Jean-Toussaint:** None. **Y. Tian:** None. **A. Sacan:** None.

Poster

220. Preclinical and Clinical Studies in Peripheral Nerve Injury and Neuropathic Pain

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 220.03/I19

Topic: D.03. Somatosensation – Pain

Support: NIH Grant NS065926

Title: An RNA-seq based, ligand-receptor interactome between 42 cell-types and subtypes of nociceptors

Authors: *A. WANGZHOU, P. R. RAY, C. A. PAIGE, G. O. DUSSOR, T. J. PRICE; Behavioral and Brain Sci., The Univ. of Texas at Dallas, Richardson, TX

Abstract: While pain researchers have been searching for novel pain drugs for decades, few have been developed. We sought to harness computational methods to identify, across the genome, how individual cell types interact with nociceptors through ligand-receptor interactions. To do this we used RNA-sequencing (RNA-seq) datasets with a focus on single-cell RNA-seq, where there has been a recent explosion in the amount of archived data available for such computational studies. Here we present a novel analysis technique for single-cell RNA-seq data that analyzes ligand and receptor interactions between 3 major subtypes of sensory neurons, including nociceptors, and 42 other cell types in the mouse. The technique is enabled by a database that catalogs all ligand-receptor pair interactions across the entire mouse genome allowing us to generate cell-type specific maps of how individual cell types express ligands that might be key for how they can communicate with sensory neurons. We are developing a web-based tool to allow users to survey these interactions to hunt for novel targets. As a demonstration of the tool, and its potential to be used across species, we used the interactome to search for potential generators of pancreatic cancer pain. The RNA-seq data of 4 paired cancer tissue vs healthy tissue from pancreatic cancer patients were acquired from TCGA database. Genes up- or down- regulated in human pancreatic cancer samples were analyzed against mouse DRG single-cell RNA-seq datasets, and validated with human DRG bulk RNA-seq data. We found key ligand-receptor interactions of Adrenomedullin (*ADM*), Ephrin B1 (*EFNB1*), and Sonic Hedgehog (*SHH*), all of which are known to promote sensitization of nociceptors. Interestingly, we also noted down-regulation of Interleukin 10 (*IL10*), and pro-Opiomelanocortin (*POMC*) both of which are involved in pain inhibition. We propose that this approach can reveal

new insight into sensory neurobiology with unprecedented resolution and that it has the potential to reveal new chronic pain mechanisms that are highly selective for specific human disease states.

Disclosures: A. Wangzhou: None. **P.R. Ray:** None. **C.A. Paige:** None. **G.O. Dussor:** None. **T.J. Price:** None.

Poster

220. Preclinical and Clinical Studies in Peripheral Nerve Injury and Neuropathic Pain

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

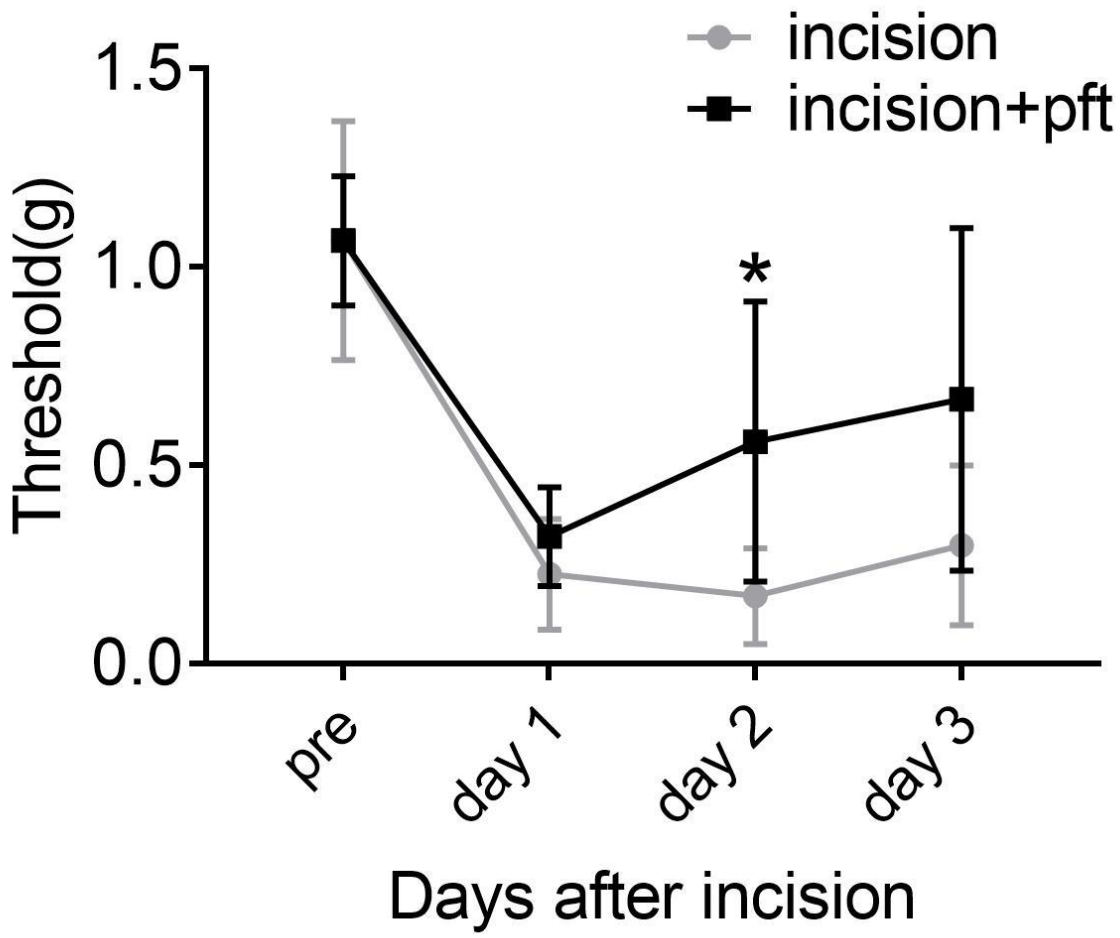
Program #/Poster #: 220.04/I20

Topic: D.03. Somatosensation – Pain

Title: p53 induction in the DRG is associated with the pain hypersensitivity after the tissue injury

Authors: *A. YAMASHITA, F. AMAYA;
Kyoto Prefectural Univ. of Med., Kyoto, Japan

Abstract: (Background) p53 is the transcriptional factor with anti-cancer property. p53 dysfunction is associated with pathophysiology of neurodegenerative diseases. We investigated role of p53 for the development of tissue injury induced pain hypersensitivity. (Methods) Male C57BL/6 (20-25g) mice were used for the experiments. Planter incision was made in left hind-paw of mice under the anesthesia isoflurane. Naïve mice were used as controls. Behavioral testing was performed to determine mechanical and thermal pain hypersensitivity. L4 and L5 DRG were taken under terminal anesthesia and proceeded for measurement of p53 mRNA expression in the DRG by RT-PCR. Separately, selective p53 inhibitor pifithrin-α (2mg/kg) was administrated intraperitoneally prior to and 2 days after the incision. (Results) Mechanical and thermal threshold were significantly decreased after the incision. In the DRG, p53 mRNA expression was significantly increased after the incision compared to control. Pifithrin-α inhibited reduction of the mechanical threshold 2 days after the incision. Pifithrin-α did not affect to the thermal pain hypersensitivity after the plantar incision. (Conclusions) p53 expression significantly increased in mice DRG after the plantar incision. Induction of p53 is associated with the development of pain hypersensitivity after the tissue injury.



Disclosures: **A. Yamashita:** None. **F. Amaya:** None.

Poster

220. Preclinical and Clinical Studies in Peripheral Nerve Injury and Neuropathic Pain

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 220.05/I21

Topic: D.03. Somatosensation – Pain

Support: KAKENHI 26670768
KAKENHI 18K16437

Title: The plasticity of spinal γ-aminobutyric acid interneurons reduces analgesic effects of morphine in neuropathic pain

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Abstract: Morphine produces powerful analgesic effects in acute pain, but its efficacy is diminished in neuropathic pain. We have shown that systemic administration of morphine increases serotonin (5-hydroxytryptamine: 5-HT) levels in the spinal cord due to activation of the descending inhibitory pathway including periaqueductal gray (PAG) and rostral ventromedial medulla system. The increase in 5-HT contributes to morphine-induced analgesia in the normal rat but attenuates that in neuropathic pain model rat through spinal 5-HT3 receptors. We hypothesized that in the setting of neuropathic pain the effect of 5-HT3 receptor in the spinal dorsal horn relatively acted as pain facilitation due to dysfunction of the inhibitory γ-aminobutyric acid (GABA) neurons. Three weeks after L5 spinal nerve ligation (SNL) with PAG cannulation, rats were subjected to behavioral testing, in vivo microdialysis of the spinal dorsal horn to determine change in concentration of 5-HT, noradrenaline, glutamate, and GABA after morphine (N=6). PAG administration of morphine (10, 100 ng) produced analgesic effects in normal rats (P=0.013, 0.004 vs saline), but not in SNL rats. PAG administration of morphine (100 ng) increased 5-HT concentration (P=0.002 vs saline in normal rats, P<0.001 in SNL rats). Intrathecal pretreatment with a 5-HT3 receptor antagonist ondansetron (3 μg), attenuated the analgesic effect of PAG administration of morphine (100 ng) in normal rats but increased the analgesic effect of morphine in SNL rats (P<0.001). Local perfusion in the spinal dorsal horn of 5-HT3 receptor agonist 2-m-5-HT (100 μM) increased GABA concentration (P<0.001 vs saline both in SNL and normal rats). Intrathecal pretreatment with a GABAA receptor antagonist bicuculline (0.03 μg), attenuated the analgesic effect of PAG administration of morphine (100 ng) in normal rats but increased the analgesic effect of morphine in SNL rats (P<0.001) and this analgesic effect was reversed by five daily intrathecal injections of a tropomyosin receptor kinase B (TrkB) antagonist K252a (2 μg/day, P<0.001). Present study demonstrated that PAG administration of morphine did not produce analgesic effect against neuropathic pain despite increased 5-HT in the spinal dorsal horn. 5-HT3 agonist increased GABA in the spinal dorsal horn and analgesic effect of morphine for neuropathic pain was increased by GABAA antagonist. These results suggest that morphine activates GABAergic interneurons in the spinal dorsal horn through 5-HT3 receptor and it relates to pain facilitation in neuropathic pain state. TrkB may contribute to the plasticity of the GABAergic interneuron in the spiral dorsal horn after nerve injury.

Disclosures: T. Hiroki: None. **T. Suto:** None. **J. Ohta:** None. **S. Saito:** None. **H. Obata:** None.

Poster

220. Preclinical and Clinical Studies in Peripheral Nerve Injury and Neuropathic Pain

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 220.06/I22

Topic: D.03. Somatosensation – Pain

Support: National Natural Science Foundation of China (NSFC #81771205)
National Natural Science Foundation of China (NSFC #91632113)
the CAMS Innovation Fund for Medical Sciences [CIFMS #2017-I2M-4-005(TW), #2017-12M-3-008(CM)].

Title: Mechanisms of evoked pain in a mouse model of chronic compression of dorsal root ganglia

Authors: *T. WANG, J. TAO, J. ZHU, C. MA;
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Abstract: The aim of the present study was to investigate the mechanisms of evoked pain to low-dose chemical stimulus in a mouse model of chronic compression of dorsal root ganglion (CCD). Chronic compression of L4 DRG was performed in mice by inserting an L-shaped stainless steel rod into the right L4 intervertebral foramina. Pain-like behaviors of mice evoked by indentation of the hairy skin at calf of right hind paw with two von Frey filaments which delivered bending force of 2g and 4g respectively were measured on pre-CCD 1d and post-CCD 1d, 3d, 5d, 7d to ensure that CCD model is conducted successfully. Different doses of capsaicin (0.1, 1, 10 μg/10 μL) were injected into the skin on the calf area of mice right hind paw respectively and subsequent behavior responses were videotaped immediately on pre-CCD 1 d and post-CCD 1, 3, 5, 7 d. The optimal concentration that leads to the most significant behavior difference after CCD was determined and was used in the following in-vivo DRG imaging studies. *Pirt*-GCaMP3 mice were given subcutaneous injection of optimal concentration of chemicals and 4 g von Frey mechanical stimuli, then calcium activities of right L4 DRG neurons were recorded *in vivo* with laser scanning confocal microscope. Immunofluorescent staining was conducted to evaluate the expression of TRPV1 in right L4 DRG from naïve and CCD mice. Mechanical behavior test showed a significant increase in the probability of paw withdrawal led by 2 g von Frey hair after on post-CCD 1d, 3d, 5d, 7d,compared to pre-CCD 1 d (n=7, P<0.01). 4 g von Frey hair caused a high probability of mice paw withdrawal on pre-CCD 1 d. There was an increase of the pain-like behavior on post-CCD 5 d (n=7, P<0.01) and little difference was observed on post-CCD 1 d, 3 d and 7 d (n=7, P>0.05). Behavioral tests showed that 1 μg/10 μL capsaicin elicited a significant difference in pain-like behaviors after CCD. In vivo calcium imaging showed an enhanced the number of activated DRG neurons to the injection of chemicals

and 4 g von Frey mechanical stimuli in CCD mice, 1 μg/10 μL capsaicin led to more activated neurons for control mice compared with CCD mice. Chronically compressed DRG neurons led to the up-regulated expression level of TRPV1 receptor and enhanced responses to low-dose of capsaicin, which produced pain hypersensitivity in the CCD mice.

Disclosures: **T. Wang:** None. **J. Tao:** None. **J. Zhu:** None. **C. Ma:** None.

Poster

220. Preclinical and Clinical Studies in Peripheral Nerve Injury and Neuropathic Pain

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 220.07/I23

Topic: D.03. Somatosensation – Pain

Support: NIH DE023846
NIH DE027731
NIH NS091296

Title: A single injection of capsaicin induces long lasting analgesia for trigeminal neuropathic pain in mice

Authors: S. WANG, J. YANG, C. BIAN, Y. GAO, F. WEI, ***M.-K. CHUNG**;
Dept. of Neural and Pain Sci., Univ. of Maryland, Sch. of Dent., Baltimore, MD

Abstract: Trigeminal neuropathic pain is a major medical problem. Injury or surgery in the trigeminal area induces debilitating persistent neuropathic pain. Since trigeminal neuropathic pain is often resistant to current pharmacotherapy, there is a pressing need to develop more efficacious treatments for trigeminal neuropathic pain with fewer side effects. TRPV1 is a Ca^{2+}-permeable ion channel that is specifically activated by the chili pepper vanilloid, capsaicin. Topical capsaicin invariably induces burning pain. Paradoxically, however, the transient pain is often followed by prolonged attenuation of the pre-existing persistent pain. Topical capsaicin is an FDA-approved treatment for post-herpetic neuralgia, providing months-long pain relief. However, the mechanisms underlying capsaicin-induced analgesia are not well understood. Despite clear therapeutic effects of capsaicin, the involvement of TRPV1 and TRPV1+ afferents in neuropathic pain is controversial. We recently reported evidence in a mouse model that TRPV1 and TRPV1+ nociceptors contributed to mechanical hyperalgesia and allodynia following neuropathy in the trigeminal area. The mechanical hyperalgesia and allodynia usually observed in mice subjected to chronic constriction injury of the infraorbital nerve (ION-CCI) was prevented by systemic pretreatment with resiniferatoxin, an ultrapotent TRPV1 agonist which desensitizes TRPV1+ afferents. We also found that local pharmacological inhibition of TRPV1 at the central terminals of primary afferents was sufficient to attenuate mechanical hyperalgesia and allodynia. In this study, we determined the analgesic effects of locally

administered capsaicin on neuropathic pain following ION-CCI and the role of TRPV1+ afferents. A single injection of capsaicin to facial skin of mice with ION-CCI led to attenuated mechanical hyperalgesia lasting for several weeks. Local capsaicin injection also decreased conditioned-place preference to lidocaine injected into trigeminal subnucleus caudalis in mice with ION-CCI. Such capsaicin-induced analgesia was associated with the capsaicin-induced ablation of nerve terminals since co-administration of capsaicin along with MDL28170, an inhibitor of calpain, abolished capsaicin-induced analgesia. Furthermore, chemogenetic inhibition of TRPV1-lineage afferents attenuated mechanical hyperalgesia following ION-CCI. These results showed that capsaicin-induced analgesia for neuropathic pain in ION-CCI is mediated by capsaicin-induced ablation of TRPV1+ nociceptor terminals, which suggest that TRPV1+ afferents contribute to the maintenance of trigeminal neuropathic pain.

Disclosures: **S. Wang:** None. **J. Yang:** None. **C. Bian:** None. **F. Wei:** None. **M. Chung:** None. **Y. Gao:** None.

Poster

220. Preclinical and Clinical Studies in Peripheral Nerve Injury and Neuropathic Pain

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 220.08/I24

Topic: D.03. Somatosensation – Pain

Support: FWF, Grant ID: DK-SPIN W1206-06
FWF, Grant ID: P25345
FWF, Grant ID: P28611
FWF, Grant ID: P30809
FP7-HEALTH, Grant ID: 602133

Title: Identification of stable reference genes for microRNA expression analysis in neuronal tissues in a mouse peripheral nerve injury model

Authors: *T. KALPACHIDOU, K. K. KUMMER, M. MITRIć, M. KRESS;
Dept. of Physiol. and Med. Physics, Med. Univ. of Innsbruck, Innsbruck, Austria

Abstract: MicroRNAs (miRNA) have emerged as major regulators of numerous biological processes in health and disease, including neuropathy. miRNA expression is commonly quantified by reverse transcription quantitative real time polymerase chain reaction (RT-qPCR), which greatly depends on normalization methods using appropriate reference genes. Different non-coding RNAs (ncRNAs) are currently used for miRNA normalization, however, there is no study identifying the optimal reference genes in animal models for peripheral nerve neuropathy. We evaluated the expression of eleven ncRNAs commonly used as reference genes in dorsal root ganglia (DRG), dorsal horn of the spinal cord (dhSC) and medial prefrontal cortex (mPFC) in the

mouse spared nerve injury model. After RT-qPCR, the stability of each candidate was determined by four different methods: BestKeeper, comparative delta-Cq method, geNorm and NormFinder. Each ncRNA was rated according to its performance in each method and an overall ranking list was compiled. We showed that the most stably expressed ncRNAs were: sno420, sno429 and sno202 in DRG; sno429, sno202 and U6 in dhSC; sno202, sno420 and sno142 in mPFC. sno55 was excluded from analysis due to its' pronounced technical variability, whereas sno135 was consistently found as one of the least stably expressed ncRNAs. We provide the first reference genes evaluation for miRNA normalization in different neuronal tissues in an animal model of peripheral nerve lesion. Our results underline the need for careful selection of reference genes for miRNA normalization in different tissues and experimental conditions. We further anticipate that our findings can be used in a broad range of nerve injury related studies, to ensure the validity and promote reproducibility in miRNA quantification.

Disclosures: T. Kalpachidou: None. **K.K. Kummer:** None. **M. Mitrić:** None. **M. Kress:** None.

Poster

220. Preclinical and Clinical Studies in Peripheral Nerve Injury and Neuropathic Pain

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 220.09/I25

Topic: D.03. Somatosensation – Pain

Support: NIH Grant T32 NS082145
NIH Grant NS082746

Title: The roles of Cav2.1 and CaMKII in hyperglycemic sensitization of peripheral sensory neurons

Authors: *R. C. EVANS[1], Y. ZHANG[2], R. GOMEZ[2], N. A. JESKE[1,2];
[1]Pharmacol., [2]Oral & Maxillofacial Surgery, UTHSCSA, San Antonio, TX

Abstract: Diabetes mellitus, a metabolic disease that is characterized by chronic hyperglycemia, is estimated to affect over 422 million people worldwide (World Health Organization, 2016). The most common complication associated with diabetes is diabetic neuropathy, marked by chronic and debilitating pain that affects more than half of diabetic patients during their lives (Callaghan et al., 2012). Multiple secondary pathologies associated with diabetes have been hypothesized to contribute to the development of this condition through the sensitization of peripheral nociceptors. However, a unifying mechanism of pathology has not been found, and represents a significant gap in knowledge. In particular, the effects of hyperglycemia on the regulation of voltage-gated calcium channels, which is a factor in other forms of peripheral neuropathy unrelated to diabetes (Li et al., 2017), has not been well evaluated. P/Q-type voltage-

gated calcium ($Ca_V2.1$) channels are expressed on pre-synaptic nerve terminals of peripheral sensory neurons and are involved in regulating neurotransmitter release (Westenbroek et al., 1998). Since deletion of the gene encoding for the α1 subunit of $Ca_V2.1$ has been shown to reduce mechanical hypersensitivity in certain neuropathic pain models (Luvisetto et al., 2006), we evaluated the role of $Ca_V2.1$ in the hyperglycemic sensitization of peripheral sensory neurons. Rat dorsal root ganglia (DRG) neurons were cultured in hyperglycemic media (25 mM glucose), which mimics rodent diabetic plasma glucose levels (Byrne et al., 2015), or in more normoglycemic media (2.5 mM glucose). Preliminary data from real-time Ca^{2+} imaging experiments demonstrate that hyperglycemia increases peak Ca^{2+} influx in cultured DRG neurons compared to normoglycemic media conditions. Additionally, treating cultured peripheral sensory neurons with a selective blocker of $Ca_V2.1$ prevented hyperglycemic sensitization, suggesting involvement of $Ca_V2.1$ channels. Furthermore, treatment with a selective inhibitor of Ca^{2+}/calmodulin-dependent kinase II (CaMKII), an enzyme that can become autonomously active under hyperglycemic conditions (Erickson et al., 2013) and is known to facilitate $Ca_V2.1$ activity (Jiang et al., 2008), also prevented hyperglycemic sensitization of cultured peripheral sensory neurons, suggesting that hyperglycemia is promoting the interaction of activated CaMKII and $Ca_V2.1$ channels. Future directions include employing a rodent model of type II diabetes to provide translational support for the *in vitro* results described above.

Disclosures: **R.C. Evans:** None. **Y. Zhang:** None. **R. Gomez:** None. **N.A. Jeske:** None.

Poster

220. Preclinical and Clinical Studies in Peripheral Nerve Injury and Neuropathic Pain

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 220.10/I26

Topic: D.03. Somatosensation – Pain

Support: NIH K08 Grant NS079482
NIH RO1 NS104295-01
NU Dixon Young Investigator Grant

Title: DRG mitochondrial homeostasis in painful diabetic neuropathy and its alteration by calcium and CXCR4 signaling

Authors: *S. HACKELBERG[1], N. D. JAYARAJ[1], D. REN[2], D. S. GEORGE[1], A. BELMADANI[2], R. J. MILLER[2], D. M. MENICHELLA[1];
[1]Neurol., [2]Pharmacol., Northwestern Univ., Chicago, IL

Abstract: Painful Diabetic Neuropathy (PDN) affects 25% of diabetes patients, making the condition one of the most frequent complications of diabetes and a major contributor to chronic pain burden. PDN's severe impact on patient quality of life and economic burden through patient

care and workforce loss calls for effective and safe treatment options, but achievement of relief is impeded as the underlying mechanisms remain unknown. Our lab has previously shown that chemokine receptor CXCR4 is necessary for the development of neuropathic pain, dorsal root ganglion (DRG) nociceptor hyperexcitability and small-fiber degeneration in the High Fat Diet (HFD) mouse model of PDN (Jayaraj et al., 2018). We further found that DRG neurons positive for the nociceptor marker $Na_v1.8$ show increased calcium responsiveness to excitatory stimuli and CXCR4 signaling. Our working hypothesis is that calcium overload induces mitochondrial dysfunction, ultimately leading to axonal degeneration. Our current studies are designed to examine mitochondrial homeostasis in the HFD model of PDN and evaluate a causal relation between calcium and CXCR4 signaling with mitochondrial function. Mitochondrial homeostasis was examined with an array of functional and morphological studies using a combination of fluorescent dyes, genetically encoded GFP derived biosensors and histology. To examine DRG Calcium physiology in vitro and in vivo, we utilized the calcium sensitive dye Fura-2, the genetically encoded calcium sensor GCaMP6 as well as knock out mice for the mitochondrial calcium uniporter (MCU). To specifically study changes in the nociceptor population, we used $Na_v1.8$-cre mice where feasible. Our preliminary results support a pivotal role of calcium signaling and show altered mitochondria morphology and function in the HFD mouse model of PDN. This contribution is expected to produce significant insights into the pathogenesis of neuropathic pain and fiber degeneration in PDN and to consequently identify novel targets essential to the development of disease modifying therapeutics.

Disclosures: S. Hackelberg: None. **N.D. Jayaraj:** None. **D. Ren:** None. **D.S. George:** None. **A. Belmadani:** None. **R.J. Miller:** None. **D.M. Menichella:** None.

Poster

220. Preclinical and Clinical Studies in Peripheral Nerve Injury and Neuropathic Pain

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 220.11/I27

Topic: D.03. Somatosensation – Pain

Support: NRF Grant 2018R1A5A2025272
NRF Grant 2019R1A2C1002555
KIOM KSN1812181

Title: Substance P is an important mediator for acupuncture effects

Authors: *Y. FAN[1], H. KIM[1], D.-H. KIM[1], Y. RYU[2], S. LEE[1], H. KIM[1], H. JANG[1], D. AHN[1], E. JEONG[1], Y. YI[1], C. YANG[1];
[1]Col. of Korean Medicine, Daegu Haany Univ., Daegu, Korea, Republic of; [2]Korean Med. Fundamental Res. Division, Korea Inst. of Oriental Med., Daejeon, Korea, Republic of

Abstract: Acupuncture has been used to treat a variety of diseases and symptoms for more than 2,500 years. Our previous study showed that acupoints are identical to neurogenic spots arising from the release of neuropeptides such as substance P (SP) from activated small diameter sensory afferents in the dermatome associated with visceral disorders. The neuropeptide SP may be an important mediator for the initiation of acupuncture effect. To explore the roles of SP in producing therapeutic effects of acupuncture, the present study investigated in a rat model of immobilization-induced hypertension whether the acupuncture effects at acupoints is closely associated with elevation of cutaneous SP level during neurogenic inflammation. When plasma extravasation from neurogenic inflammation was examined by exploring the leakage of intravenously injected Evans blue dye (EBD) to the skin, extravasated EBD began to appear at acupoints on the wrist, gradually accumulated and fully saturated within 15 min after EBD injection. Significant increase of SP over acupoint in hypertensive rats were observed, compared to control. Injection of either SP or capsaicin produced anti-hypertensive effects, which was reversed by injecting of an SP antagonist into acupoints on the wrist. Moreover, single fiber recording displayed that local injection of SP into acupoint increased the sensitivity of A- and C-fibers in response to acupuncture stimulation. In addition, the rate of discharge of wide-dynamic response (WDR) neurons in spinal cord significant increased following intradermal SP treatment in naive rats but decreased following intradermal SP antagonists in hypertensive rats. Therefore, our findings suggest that SP is an important mediator in the development of acupuncture effects.

Disclosures: Y. Fan: None. **H. Kim:** None. **D. Kim:** None. **Y. Ryu:** None. **S. Lee:** None. **H. Kim:** None. **H. Jang:** None. **D. Ahn:** None. **E. Jeong:** None. **Y. Yi:** None. **C. Yang:** None.

Poster

220. Preclinical and Clinical Studies in Peripheral Nerve Injury and Neuropathic Pain

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 220.12/I28

Topic: D.03. Somatosensation – Pain

Title: Effects of external low intensity focused ultrasound on electrophysiological changes *in vivo* in a rodent model of common peroneal nerve injury

Authors: A. HELLMAN[1], T. MAIETTA[1], K. BYRAJU[1], P. NEUBAUER[2], E. WILLIAMS[2], C. BURDETTE[2], D. S. SHIN[1], G. GHOSHAL[2], J. QIAN[3], J. NALWALK[1], *J. G. PILITSIS[1]; [1]Albany Med. Col., Albany, NY; [2]Acoustic Medsystems, Savoye, IL; [3]Albany Med. Ctr., Albany, NY

Abstract: Non-invasive treatment methods for neuropathic pain caused by nerve injury are currently limited. We have previously determined that external low intensity focused ultrasound (liFUS) improves nociceptive sensory thresholds when applied to the dorsal root ganglia (DRG) in common peroneal nerve injury (CPNI), chronic migraine, and chemotherapy induced

neuropathy models of chronic pain in rats. Here we assess how external liFUS modulation of the L5 DRG affects behavioral responses and sensory nerve action potentials (SNAPs) in a CPNI model. Rats were assessed for mechanical and thermal pain responses (pre-liFUS) following CPNI surgery using Von Frey filaments and the hot plate test, respectively. They were then treated with liFUS (8W for 3 minutes with 11MHz pulsed at 38Hz with a period of 90ns, and a pulse width of 13ms) and pain responses were re-tested 24 hours later (post-liFUS). Significant improvements in mechanical and thermal sensory thresholds ($p < 0.0001$, $p = 0.02$, respectively) were seen post-liFUS treatment, indicating a reduction in pain sensitivity. SNAPs of the injured common peroneal nerve (CPN) were also monitored pre- and post-liFUS. Prior to liFUS treatment, animals who received CPNI surgery had significantly longer SNAP latencies when compared to the control sham CPNI surgery animals ($p=0.0003$). LiFUS induced significant reductions in CPN SNAP latency in both CPNI liFUS and sham CPNI liFUS cohorts, for up to 35 minutes post treatment ($p = 0.03$ and $p = 0.02$; at 35 minutes post liFUS for the CPNI liFUS cohort and at 30 minutes post liFUS for the sham CPNI liFUS cohort respectively). No changes were seen in SNAP amplitude in any cohort at any time tested. In addition, no evidence of neuronal degeneration was seen in the DRG 24 hours after liFUS treatment, suggesting that liFUS did not damage the tissue being modulated. This study shows that external application of liFUS to the L5 DRG, at a dose capable of reducing both mechanical and thermal nociceptive thresholds, results in significant reduction in CPN SNAP latency, but not in SNAP amplitude. This is the first *in vivo* study of the impact of liFUS on peripheral nerve electrophysiology in a model of chronic pain.

Disclosures: **A. Hellman:** None. **T. Maietta:** None. **K. Byraju:** None. **P. Neubauer:** A. Employment/Salary (full or part-time):; Acoustic Medsystems. **E. Williams:** A. Employment/Salary (full or part-time):; Acoustic Medsystems. **C. Burdette:** A. Employment/Salary (full or part-time):; Acoustic Medsystems. **D.S. Shin:** None. **G. Ghoshal:** A. Employment/Salary (full or part-time):; Acoustic Medsystems. **J. Qian:** None. **J. Nalwalk:** None. **J.G. Pilitsis:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH 2RO1CA166379-06, 1R43NS107076-01A1, Medtronic, Boston Scientific, Abbott, Nervo, Jazz Pharmaceuticals, GE Global Research. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Acoustic Medsystems. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aim Medical Robotics, Karuna. F. Consulting Fees (e.g., advisory boards); Aim Medical Robotics, Karuna.

Poster

220. Preclinical and Clinical Studies in Peripheral Nerve Injury and Neuropathic Pain

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 220.13/I29

Topic: D.03. Somatosensation – Pain

Support: MINECO SPAIN (SAF2016-77585-R)
Universidad de Alcala (CCGP2017-BIO/037)
LB helds a Scholarship (Ministerio Educación, Cultura y Deporte, Spain)

Title: Expression of pSTAT3 after peripheral nerve damage: Injured and non-injured afferents profile in neuropathic pain models

Authors: L. BERNAL[1], E. CISNEROS[2], *C. ROZA[1];
[1]Univ. De Alcala, Alcala de Henares, Spain; [2]Univ. de Alcala/CUNIMAD, Alcala de Henares, Spain

Abstract: Spontaneous pain, a symptom commonly reported by patients with peripheral neuropathies, is produced by aberrant spontaneous discharges fired by peripheral afferents. Animal studies performed in our laboratory showed that, in clear contrast with total axotomy, partial nerve damage increases the incidence of C-fibers with stable and relatively high rates of spontaneous activity in both axotomized and intact units and prompted a functional cross-talk between them. Detection of axonal at the site of injury triggers changes in the cell bodies in order to switch the neuron phenotype from a transmitting to a regeneration mode. However, it is unclear if such changes might also occur in the uninjured afferents or whether they depend on the injury type. Here we evaluated the regenerative status of retrogradely labeled dorsal root ganglion (DRG) neurons in different models of neuropathic pain.
Spared nerve injury -SNI- or total axotomy -TA- of the left sciatic nerve was performed in mice under deep anaesthesia and red fluorescence Retrobeads (RB) were applied at the severed stump. Two weeks post-surgery, mice were perfused intracardially and the left L4 DRG was extracted, post-fixed and made transparent following an inCLARITY protocol that allows immunostaining in whole mounts. The samples were incubated with primary antibodies for pSTAT3 (signal transducer and activator of transcription 3) during 5 days and secondary antibodies for 2 days.The whole DRGs were mounted with Mowiol and observed under a Leica TCS SP5 confocal microscope. Images were analyzed using Fiji software, for quantification an intensity fluorescence threshold was set for all samples of each experiment
A total of 215 (SNI) and 487 (TA) neurons were counted in L4 ganglia, of which 15.8 and 31.2 % respectively were labelled with RB. Regarding pSTAT3 expression, 45 and 58.7% of the neurons were positive in the SNI and TA models, respectively. No pSTAT3 signal was detected in control mice (n = 2 mice). The proportion of injured cells (RB^+) expressing pSTAT3 was

larger in SNI than in TA mice (58.8 vs 47.4 %) indicating that the regenerating response is enhance in partially damaged nerves. Finally, pSTAT3 was also present in RB- neurons, indicating that regenerative signaling also occurs in "putative intact" fibers.
We propose that the hyperexcitability of peripheral axons after nerve injury is trigger by upregulation of transcription factors involved in the stress-related response in both axotomized and "putative intact" axons, and this depend on the degree of damage. It is likely that a less aggressive and extended environment developed after partial injury favor regeneration in axotomized neurons.

Disclosures: L. Bernal: None. **E. Cisneros:** None. **C. Roza:** None.

Poster

220. Preclinical and Clinical Studies in Peripheral Nerve Injury and Neuropathic Pain

Location: Hall A

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Program #/Poster #: 220.14/I30

Topic: D.03. Somatosensation – Pain

Support: NIH Grant R01NS065926
NIH Grant R01NS098826

Title: The cellular basis of protease activated receptor type 2 evoked mechanical and affective pain

Authors: *A. AHMAD[1], S. HASSLER[1], M. KUME[1], S. SHIERS[1], J. MWIRIGI[1], A. WANGZHOU[1], D. NAIK[1], G. DUSSOR[1], J. VAGNER[2], S. BOITANO[3], T. PRICE[1];
[1]Brain and Behavioral Sci., Univ. of Texas At Dallas, Richardson, TX; [2]Bio5 Res. Inst., [3]Dept. of Physiol., Univ. of Arizona, Tuscon, AZ

Abstract: Protease activated receptor type 2 (PAR2) has long been implicated in inflammatory and visceral pain but the cellular basis of PAR2-evoked pain has not been delineated. While many studies have attributed PAR2-evoked pain to sensory neuron expression, RNA-sequencing experiments are ambiguous on detection of *F2rl1* mRNA. Moreover, many pharmacological tools for PAR2 have been shown to be non-specific as they also act on the Mrg family of g-protein coupled receptors (GPCRs) that are highly enriched in sensory neurons. We sought to bring clarity to the cellular basis of PAR2 pain. We developed a PAR2 conditional mutant mouse by loxp targeting of exon 2 of the *F2rl1* gene and specifically deleted PAR2 in all sensory neurons using the *Pirt*^{Cre} mouse line. Our behavioral findings show that PAR2-evoked mechanical hyperalgesia and facial grimacing, but not thermal hyperalgesia, is completely dependent on PAR2 expression in sensory neurons in male and female mice. F2rl1 mRNA is expressed in a discrete population (~4%) of sensory neurons that also express the *Nppb* and *IL31ra* genes. This cell population has previously been implicated in itch, but our work shows

that PAR2 activation in these cells causes pain, but not itch, behaviors. Our findings clarify the mechanism through which proteases, like tryptase and elastase, cause pain via PAR2 activation in a small subset of nociceptors.

Disclosures: **A. Ahmad:** None. **S. Hassler:** None. **M. Kume:** None. **S. Shiers:** None. **J. Mwirigi:** None. **A. Wangzhou:** None. **D. Naik:** None. **G. Dussor:** None. **J. Vagner:** None. **S. Boitano:** None. **T. Price:** None.

Poster

220. Preclinical and Clinical Studies in Peripheral Nerve Injury and Neuropathic Pain

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 220.15/I31

Topic: D.03. Somatosensation – Pain

Support: NINDS R01NS098772
NIDA R01DA042852

Title: CRMP2: Roles pre- and post-synaptic spinal neurotransmission?

Authors: *L. BOINON[1], J. YU[1], A. MOUTAL[1], A. CHEFDEVILLE[1], D. L. FEINSTEIN[2], R. KHANNA[1,3,4,5];
[1]Univ. of Arizona, Tucson, AZ; [2]Anesthesiol., Univ. of Illinois at Chicago, Chicago, IL; [3]The Ctr. for Innovation in Brain Sciences, The Univ. of Arizona Hlth. Sciences, Tucson, Arizona, Tucson, AZ; [4]Regulonix Holding Inc., Tucson, Arizona, Tucson, AZ; [5]BIO5 Institute, Univ. of Arizona, Tucson, AZ

Abstract: The collapsin response mediator protein 2 (CRMP2) has emerged as a central node in assembling nociceptive signaling complexes involving voltage-gated ion channels. Concerted actions of post-translational modifications, phosphorylation and SUMOylation, of CRMP2 contribute to regulation of pathological pain states. We previously reported convergent regulation of Cav2.2 and Nav1.7 functions by CRMP2; this occurred at pre-synaptic sites in the dorsal horn of the spinal cord thereby regulating spinal neurotransmission. The function of CRMP2 at post-synaptic sites remains unknown. Here, we used an *in vivo* CRMP2 knockdown (siRNA) strategy in rats to ask if CRMP2 could play a role in post-synaptic transmission in the dorsal horn of the spinal cord. We recorded spontaneous excitatory postsynaptic currents (sEPSCs) and found that CRMP2 knockdown decreased both the frequency and the amplitude of sEPSCs. In parallel, we utilized a transgenic CRMP2$^{flox/flox}$ mouse transduced with a CaMKII-cre virus to delete CRMP2 from neurons within the dorsal root ganglion and the spinal cord. Similar to the CRMP2 knockdown approach, the frequency and amplitude of sEPSCs was decreased in neurons without CRMP2. Notably, deleting CRMP2 in astrocytes (using a GFAP-Cre virus) had no effect on sEPSCs. These results demonstrate that CRMP2 plays both a pre- as well as a post-

synaptic role in excitatory neurotransmission in the dorsal horn of the spinal cord. We are currently exploring whether CRMP2 manipulation has any effects on spontaneous inhibitory postsynaptic currents. Our novel data open novel routes for understanding the role of CRMP2 in pain by highlighting an unknown role in regulating post-synaptic spinal neurotransmission.

Disclosures: L. Boinon: None. **J. Yu:** None. **A. Moutal:** None. **A. Chefdeville:** None. **D.L. Feinstein:** None. **R. Khanna:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Regulonix Holding Inc..

Poster

220. Preclinical and Clinical Studies in Peripheral Nerve Injury and Neuropathic Pain

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 220.16/I32

Topic: D.03. Somatosensation – Pain

Support: TTUHSC Start Up Fund

Title: Role of histone acetylation in diabetic neuropathy

Authors: *M. C. CHATTOPADHYAY, V. S. THAKUR;
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Abstract: Peripheral sensory neuropathy is one of the most common complications of diabetes. Accumulating evidence suggests that chronic low-grade inflammation is involved in the pathogenesis of the disease. Modulation of histone deacetylation by Class I histone deacetylase (HDAC) specifically HDAC1, HDAC2, and HDAC3 play an important role in the transcriptional regulation of specific genes required for differentiation, survival and the function of neural cells. A growing body of literatures suggest that inhibition of HDAC activity provides relief from experimental neuropathic pain and retinal nerve degeneration. We hypothesize that hyperglycemia causes changes in histone acetylation and release of inflammatory mediators in the peripheral nervous system of diabetic animals with painful neuropathy; therefore blocking this increase will prevent or delay the development of neuropathy. In this study, we investigated whether Class I HDAC inhibition reduces neuroinflammation and neuropathic pain by evaluating the changes in inflammatory mediators and histone modifications in the dorsal root ganglia (DRG) and spinal cord dorsal horn neurons of diabetic animals treated with HDAC inhibitor as well as compared the changes in pain behavior with treatment. Type 2 diabetic (T2D) animals with pain were treated with Class I HDAC inhibitor, FK228 1mg/kg; I.P. twice a week for 3 weeks. T2D animals demonstrated significant changes in thermal hyperalgesia manifested by a decrease in withdrawal latency to heat after 6 weeks of diabetes and also exhibited marked increases in HDAC1, HDAC2, HDAC3, IL1β, TLR4, CXCR4 and alteration H3 acetylation as

determined by the Western blot analysis and immunohistochemistry. Our results show that animals treated with FK228 had significant alleviation in thermal hyperalgesia along with changes in histone acetylation and expression of inflammatory mediators. This primary study suggests that Class 1 HDACs play an important role in the inflammatory aspect of the painful neuropathy in type 2 diabetic animals and may provide a novel treatment approach for this difficult-to-treat complication of diabetes.

Disclosures: M.C. Chattopadhyay: None. **V.S. Thakur:** None.

Poster

220. Preclinical and Clinical Studies in Peripheral Nerve Injury and Neuropathic Pain

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 220.17/I33

Topic: D.03. Somatosensation – Pain

Support: NIH Grant NS098826

Title: The potent protease-activated receptor 2 (PAR2) antagonist C781 inhibits PAR2 mediated mechanical hypersensitivity and hyperalgesic priming in mice

Authors: *M. KUME[1], S. BOITANO[2,3], J. VAGNER[3], G. DUSSOR[1], T. PRICE[1];
[1]Sch. of Behavioral and Brain Sci., The Univ. of Texas at Dallas, Richardson, TX; [2]Arizona Resp. Ctr. and Dept. of Physiol., [3]The BIO5 Collaborative Res. Inst., Univ. of Arizona, Arizona, AZ

Abstract: Protease-activated receptor 2 (PAR2) is a G-protein coupled receptor linked to numerous pathologies, including acute and chronic pain. Its activation by endogenous or exogenous serine-proteases is known to cause neuronal plasticity and chronic pain based on a hyperalgesic priming model in mice. Previously, we discovered and evaluated a PAR2 antagonist called C391 that blocked PAR2 signaling *in vitro* and *in vivo* via Ca^{2+} and MAPK/ERK pathways. However, we have developed a more potent and drug-like PAR2 antagonist called C781. We evaluated the antagonistic effects of C781 using a highly specific PAR2 agonist, 2-aminothiazol-4-yl-LIGRL-NH2 (2AT), which evokes long-lasting acute mechanical hypersensitivity when injected into the paw as well as hyperalgesic priming following a subthreshold dose injection of prostaglandin E2 (PGE2). We saw that both a local co-injection (1 μg) and systemic dosing of C781 (intraperitoneal dosing at 30, 10 and 3 mg/kg) blocked acute mechanical hypersensitivity as well as hyperalgesic priming from PAR2 activation via 2AT. Future experiments will test C781's ability to block mechanical hypersensitivity and hyperalgesic priming effects of compound 48/80, neutrophil elastase, and carrageenan injections into the paw. These 3 stimuli are thought to cause pain hypersensitivity via PAR2 activation but

involve protease-mediated mechanisms, as opposed to 2AT which mimics the tethered ligand of PAR2 that is revealed by protease cleavage.

Disclosures: M. Kume: None. **S. Boitano:** None. **J. Vagner:** None. **G. Dussor:** None. **T. Price:** None.

Poster

220. Preclinical and Clinical Studies in Peripheral Nerve Injury and Neuropathic Pain

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 220.18/I34

Topic: D.03. Somatosensation – Pain

Support: Canadian Breast Cancer Foundation

Title: Improved white matter health following mindfulness training for neuropathic pain in women treated for breast cancer

Authors: *A. LEEMING[1], L. FANG[2], T. HATCHARD[3], O. MIODUSZEWSKI[3], Y. SHERGILL[4], E.-L. KHOO[4], P. POULIN[2], A. SMITH[3];
[1]Clin. Psychology, [3]Psychology, [2]Univ. of Ottawa, Ottawa, ON, Canada; [4]McMaster Univ., Hamilton, ON, Canada

Abstract: In 2017, an estimated 26,300 women were diagnosed with breast cancer in Canada, which represented 25% of all new cancer cases in women that year (Canadian Cancer Society, 2019). Following treatment, many women continue to suffer from chronic and painful symptomologies including chronic neuropathic pain (CNP). CNP is defined as a discrete set of pain symptoms caused by a primary lesion or dysfunction of the nervous system including postsurgical nerve and tissue damage and inflammation lasting 3 months beyond normal healing time (Merskey & Bogduk, 1994; Jung et al, 2003). CNP affects 20-50% of women following breast cancer treatment (Bokhari & Sawatzky, 2009). A burgeoning drug-free holistic intervention, mindfulness-based stress reduction (MBSR), is a form of meditation characterized by paying attention to the present moment with openness, curiosity, and acceptance. It functions by refocusing the mind and increasing awareness of one's external surroundings and inner sensations, allowing the individual to step back and reframe experiences. Several studies have shown promising outcomes on pain and mood symptoms, anxiety and depression, and pain-related drug use (Hilton et al., 2017). The main objective of this study was to compare cerebral white matter health in women who had undergone breast cancer treatment and were suffering from CNP before and after an 8-week MBSR course (n=13) with a no-treatment control group (n=10). MRI diffusion tensor imaging (DTI) was acquired using a fluid attenuated inversion recovery (FLAIR) double-refocused spin echo sequence. Pre and Post-MBSR results were compared with repeated measures t-tests for each group and between groups. Although there

were no between group differences (possibly due to small sample size), there was significantly increased fractional anisotropy (FA) (a measure of white-matter tract microstructural integrity) from pre- to post-treatment in the MBSR group. Affected areas were mainly located in the left subcortical regions including the external capsule, uncinate fascinate, amygdala, and hippocampus. Additionally, there was increased FA in left sagittal stratum, including the inferior fronto-occipital fasciculus, the inferior longitudinal fasciculus, and the posterior thalamic radiation. No decreased FA was found during the post scan in the MBSR group. These results suggest that after only an 8-week MBSR program there can be alterations in the integrity of the white matter tracts in women with CNP following breast cancer treatment. These results provide empirical evidence of a change in the brain with MBSR and give hope for a drug-free method of easing the struggle of CNP in these women.

Disclosures: A. Leeming: None. **L. Fang:** None. **T. Hatchard:** None. **O. Mioduszewski:** None. **P. Poulin:** None. **A. Smith:** None. **Y. Shergill:** None. **E. Khoo:** None.

Poster

220. Preclinical and Clinical Studies in Peripheral Nerve Injury and Neuropathic Pain

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 220.19/I35

Topic: D.03. Somatosensation – Pain

Support: Medical Research Council grant G1002183
Medical Research Council grant MR/K021303/1
Wellcome Trust grant 080097
National Natural Science Foundation of China grant 31872788
National Natural Science Foundation of China grant 91732108

Title: Repressor element 1-silencing transcription factor is necessary and sufficient for the development of chronic pain

Authors: F. ZHANG[1], S. GIGOUT[2], Y. LIU[1], Y. WANG[1], H. HAO[1], N. J. BUCKLEY[3], H. ZHANG[1], I. C. WOOD[2], ***N. GAMPER**[2];
[1]Pharmacol., Hebei Med. Univ., Shijiazhuang, China; [2]Fac. of Biol. Sci., Univ. of Leeds, Leeds, United Kingdom; [3]Psychiatry, Univ. of Oxford, Oxford, United Kingdom

Abstract: Chronic pain is an unmet clinical problem with immense individual, societal and economic impact. Pathological over-activity of the peripheral damage-sensing (nociceptive) afferents is one of the major drivers of the chronic pain. This overexcitable state of nociceptors is, in part, produced by the large-scale dysregulation of genes controlling nociceptive neuron excitability. Despite intense research, a unifying theory behind neuropathic remodelling is lacking. Here we show that transcriptional suppressor, repressor element 1-silencing

transcription factor (REST, NRSF), is necessary and sufficient for the development of hyperalgesia following chronic nerve injury or inflammation. Viral overexpression of REST in mouse DRG *in vivo* induced mechanical and thermal hyperalgesia. Sensory neuron specific, inducible knock-out of *Rest* in mice prevented the development of chronic hyperalgesia in three different chronic pain models. Genetic deletion of *Rest* or pharmacological inhibition of REST activity reverted injury-induced hyperalgesia. Moreover, *in vivo* viral overexpression of REST in the same neurons, in which *Rest* was genetically deleted, restored neuropathic hyperalgesia. Finally, sensory neuron specific *Rest* knockout prevented injury-induced downregulation of REST target genes in DRG neurons. This work identified REST as a major epigenetic regulator of peripheral somatosensory neuron remodelling leading to chronic pain. The findings might help to develop novel therapeutic approaches to combat chronic pain.

Disclosures: F. Zhang: None. **S. Gigout:** None. **Y. Liu:** None. **Y. Wang:** None. **H. Hao:** None. **N.J. Buckley:** None. **H. Zhang:** None. **I.C. Wood:** None. **N. Gamper:** None.

Poster

220. Preclinical and Clinical Studies in Peripheral Nerve Injury and Neuropathic Pain

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 220.20/I36

Topic: D.03. Somatosensation – Pain

Support: NIH grants NS101880
GM120844

Title: Nerve injury diminishes cannabinoid analgesia through G9a-mediated transcriptional repression of CB1 receptors in primary sensory neurons

Authors: Y. LUO, ***J. ZHANG**, L. CHEN, S.-R. CHEN, H. CHEN, H.-L. PAN;
Anesthesiol. & Perioperative Med., Univ. of Texas MD Anderson Cancer Ctr., Houston, TX

Abstract: Chronic neuropathic pain remains a major therapeutic challenge. Cannabinoids can produce potent analgesic effects through stimulation of cannabinoid type-1 (CB1) receptors. However, the epigenetic mechanism regulating the expression of CB1 receptors in neuropathic pain is unclear. G9a (encoded by the *Ehmt2* gene), a histone 3 at lysine 9 (H3K9) methyltransferase, is a key chromatin regulator responsible for gene silencing. In this study, we determined the expression of CB1 receptors in the dorsal root ganglia (DRG) and the role of G9a in regulating CB1 receptor-mediated analgesic effects in an animal model of neuropathic pain. Real-time PCR and immunoblotting analysis showed that the expression level of CB1, but not CB2, receptors in the DRG was persistently repressed after spinal nerve ligation injury in rats. Chromatin immunoprecipitation-PCR assay indicated that the suppressed CB1 receptor expression was associated with an increase in H3K9me2, a repressive histone mark catalyzed by

G9a, at the promoter region of CB1 receptors. G9a inhibition in nerve-injured rats not only reversed the expression level of CB1 receptors in the DRG but also potentiated the analgesic effect of ACEA, a specific CB1 receptor agonist, on pain hypersensitivity induced by nerve injury. Furthermore, in mice lacking G9a in DRG neurons, nerve injury failed to reduce CB1 receptor expression in the DRG and the analgesic effect of ACEA. In addition, G9a inhibition or genetic knockout normalized nerve injury-induced reduction in the inhibitory effect of ACEA on synaptic glutamate release from primary afferent nerves. Together, our findings indicate that nerve injury downregulates CB1 receptors in primary sensory neurons and diminishes the cannabinoid analgesic effect on neuropathic pain. G9a is responsible for epigenetic repression of CB1 receptors in primary sensory neurons in neuropathic pain.

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Poster

220. Preclinical and Clinical Studies in Peripheral Nerve Injury and Neuropathic Pain

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 220.21/DP08/I37

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: D.03. Somatosensation – Pain

Support: NIH NCATS R42-TR001270

Title: Rapid *in vitro* detection of drug-induced peripheral neuropathy through electrophysiological and histological analysis of 3D microphysiological "nerve-on-a-chip" cultures

Authors: *K. J. POLLARD[1], H. R. KARIMIAN[1], L. J. CURLEY[4], P. KORDJAMSHIDI[2], M. J. MOORE[4,1,3];
[1]Biomed. Engin., [2]Computer Sci., [3]Tulane Brain Inst., Tulane Univ., New Orleans, LA; [4]AxoSim Inc, New Orleans, LA

Abstract: Drug-induced peripheral neuropathy (DIPN) is a common side effect that results in late-stage failure of newly developed pharmaceutical compounds and compromises the efficacy of drug-based clinical treatment. Early detection of DIPN will both streamline the drug discovery pipeline and limit the number of patients receiving pharmaceutical treatment resulting in previously unidentified neurotoxicity. Here we present a microphysiological “nerve-on-a-chip” platform for early detection of DIPN that uniquely combines the cost-effective, high-throughput workflow of conventional *in vitro* model systems with relevant physiological outputs of *in vivo* model systems. Explanted embryonic rat dorsal root ganglion tissue is grown in a 3-dimensional

bioengineered hydrogel environment that encourages regrown nerve fibers to assume their native tissue morphology and cytoarchitecture. This permits *in vitro* examination of the native functional and structural properties of peripheral nerves, including long-distance conduction of compound action potentials (CAPs) and successful myelination of peripheral nerve fibers. In the present work, “nerve-on-a-chip” cultures were matured for 28-32 days and then treated for 7 additional days with a panel of neurotoxic compounds. After treatment, nerve health was assessed by electrophysiological analysis of CAP conduction and nerve tissue was prepared for transmission electron microscopic (TEM) analysis. Customized machine-learning algorithms were used to extract an array of amplitude and conduction velocity (CV)-related descriptive metrics. Treatment with the chemotherapeutic paclitaxel, the antipsychotic chlorprothixene, and the antibiotic metronidazole resulted in balanced reductions of CAP amplitude and CV across all recording configurations. The chemotherapeutic cisplatin disproportionately reduced amplitude and CV when stimulating most proximal to the recording site while the chemotherapeutic vincristine disproportionately reduced amplitude and CV when stimulating most distally. The immunosuppressant teriflunomide showed a specific reduction in the amplitude of proximal recordings while the anticonvulsant phenytoin specifically reduced the CV of the most distal recordings. These results will be compared with histological analysis of nerve health upon completion of TEM imaging. We conclude that this peripheral “nerve-on-a-chip” model is a robust and versatile method for high-throughput, functionally relevant screening of unknown compounds for neurotoxicity.

Disclosures: **K.J. Pollard:** None. **H.R. Karimian:** None. **L.J. Curley:** A. Employment/Salary (full or part-time):; AxoSim Inc. **P. Kordjamshidi:** None. **M.J. Moore:** A. Employment/Salary (full or part-time):; AxoSim Inc.

Poster

220. Preclinical and Clinical Studies in Peripheral Nerve Injury and Neuropathic Pain

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 220.22/I38

Topic: D.03. Somatosensation – Pain

Title: Chronic fructose leads to insulin resistance and neuropathic pain in rats

Authors: *G. GARCÍA[1], E. J. GUTIÉRREZ-LARA[1], V. GRANADOS-SOTO[2], J. MURBARTIÁN[1];
[1]Dept. de Farmacobiología, [2]Neurobio. of Pain Laboratory, Dept. de Farmacobiología, Cinvestav, Mexico City, Mexico

Abstract: Peripheral neuropathy is one of the main complications of diabetes and affects nerve fibers of the peripheral nervous system in at least 50% of diabetic patients. It is proposed that peripheral neuropathy is caused by hyperglycemia, but the underlying mechanisms are not

completely understood. Nonetheless, there is evidence that peripheral neuropathy is also present in patients with metabolic syndrome and pre-diabetes, without hyperglycemia. This suggests that this disorder begins before high glucose blood levels are established. In this study, we developed an insulin resistance model induced by 15% fructose in drinking water for 16 weeks as a model of neuropathic pain in rats. We used a behavioral and molecular approach to understand how insulin resistance induces pain before diabetes is established. Chronic administration of fructose slightly enhanced blood glucose levels, but increased insulin plasma levels and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) index. Moreover, chronic fructose reduced 50% withdrawal threshold in a time-dependent fashion in both paws, which was interpreted as tactile allodynia and induced hyperalgesia (to 0.5% formalin). Systemic administration of gabapentin (50-200 mg/kg, po) and metformin (50-200 mg/kg, po), but not diclofenac (1-10 mg/kg, po), reversed in a dose-dependent manner fructose-induced tactile allodynia. Chronic fructose enhanced activating factor transcription 3 (ATF3), but not caspase-3 and α2δ-1 subunit, protein expression in individual L4 and L5 dorsal root ganglia (DRG) and sciatic nerve. Moreover, chronic fructose increased anoctamin-1 and ASIC3, whereas it reduced insulin receptor-β, α_5GABA_A receptors and TASK-3 channels protein expression in DRG and sciatic nerve. In contrast, fructose did not change TRPV1 channel protein expression in these sites. Treatment with metformin for 4 weeks reversed some of the fructose-induced changes in protein expression. Taken together, these data suggest that insulin resistance induced by fructose produces neuropathic-like pain and changes in protein expression of channels associated with the nociceptive processing in DRG and sciatic nerve.

Disclosures: G. García: None. **E.J. Gutiérrez-Lara:** None. **V. Granados-Soto:** None. **J. Murbartián:** None.

Poster

220. Preclinical and Clinical Studies in Peripheral Nerve Injury and Neuropathic Pain

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 220.23/I39

Topic: D.03. Somatosensation – Pain

Support: NIH/NCI grant R43CA206796

Title: Biomarkers for painful vs painless peripheral neuropathy: Diode laser fiber-selective stimulation (DLss) vs. traditional quantitative sensory testing (QST)

Authors: *M. I. NEMENOV[1,2], S. HAROUTOUNIAN[3];
[1]Lasmed LLC, Mountain View, CA; [2]Anesthesia, Stanford Univ., Palo Alto, CA; [3]Anesthesia, Washington Univ., Saint Louis, MO

Abstract: Mechanisms behind painful peripheral neuropathies (PPN) are still unclear and biomarkers are not available. Traditional quantitative sensory tests (QSTs) do not separate painful and painless neuropathy patients. QSTs likelymeasure responsiveness of (the partially depleted) epidermal C and Aδ[D1] nociceptive fibers to cutaneous thermal and mechanical stimulation. They are unable to provide measurements representing ongoing neuropathic pain[1]. We developed a diode laser, fiber-type specific, selective stimulation (DLss) technique that selectively accesses and simulates either C or Aδ fibers [2]. DLss radiation penetrates deep into the skin, homogeneously heating superficial and subepidermal skin layers, thereby allowing access to superficial and/or deep fibers. We previously demonstrated that painful peripheral neuropathy affects C and Aδ fibers differently in patients with diabetic and chemotherapy-induced neuropathies. Spontaneously active C fibers are sensitized to heat in PPN patients and the ratio of Aδ/C fiber pain threshold is significantly different between PPN patients and healthy controls [2-4]. DLss-stimulation depth allows for access to mechano-insensitive small fibers that are likely contributing to neuropathic pain in peripheral neuropathies [5]. Here, we applied the DLss Aδ and C fiber tests to a painful chemotherapy-induced peripheral neuropathy (CIPN) patient group (N=13) and a control group of cancer patients who received the same type and dose of chemotherapy but did not develop painful neuropathy (N=7). To qualify for the painful CIPN group, a score >4 on DN4 questionnaire and reported pain severity > (=) 3 on 0-10 numerical rating scale were required. DLss stimulation was applied to the dorsum of the foot to identify pain and detection thresholds for C and Aδ fibers. Subsequently, QST was performed to determine warmth and cold detection thresholds, heat and cold pain thresholds, and mechanical and vibration detection thresholds. While traditional QST measures were not different between groups, the Aδ/C ratio of detection thresholds in the painful CIPN (2.33) and control group (1.58) were significantly different ($P < 0.04$, t-test).These preliminary data suggest that DLss may serve as a test for differentiating painful and painless peripheral neuropathy. 1. Kleggetveit et al. Pain. 2012;153(10):2040-7.2. Moeller-Bertram et al. Pain Medicine 2013; 14 (3): 417-421.3. Nemenov, Namer, et al. SFN Abstr., Chicago, IL 2015. 4.Nemenov, Backonja.SFN Abstr., Chicago, IL 2015. Nemenov et al. *15 World Congress on Pain*, Boston, USA, 2018

Disclosures: M.I. Nemenov: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); LasMed. **S. Haroutounian:** None.

Poster

220. Preclinical and Clinical Studies in Peripheral Nerve Injury and Neuropathic Pain

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 220.24/I40

Topic: D.03. Somatosensation – Pain

Support: National Natural Science Foundation of China (NFSC#81671086)

SUSTech Foundation #Y01416102

Title: Gut microbiota in chronic pain caused by peripheral nerve injury, cis-platin chemotherapy, and diabetes in mice

Authors: *P. MA[1,2], C.-J. QIU[1], G.-H. WU[1], R.-F. MO[1], Y.-R. ZHAO[1], X.-J. SONG[1]; [1]SUSTech Ctr. for Pain Med. and Med. Sch., Southern Univ. of Sci. and Technol., Shenzhen, China; [2]Div. of Biol. and Biomed. Sci., Washington Univ. in St. Louis, St. Louis, MO

Abstract: It has been demonstrated that the gut microbiota can produce significant impact on neuronal functions and neurological diseases and disorders including Alzheimer's disease, multiple sclerosis, autism, and pain. Chronic pain caused by different forms of injury, stress, diseases, or treatment, for instance, nerve injury, chemotherapy, diabetes, and cancer etc., may exhibit similar or dissimilar painful symptoms but have distinct pathogenesis. In this study we investigated the role of gut microbiota in the development of pain after sciatic nerve injury (CCI model), cis-platin chemotherapy, and diabetes (STZ model) in C57BL/6 mice. The gut microbiota was depleted by continuous feeding of antibiotics cocktail (g/L, vancomycin 0.5, ampicillin 1.0, neomycin 1.0, and metronidazole 1.0 dissolved in water). Antibiotics treatment significantly decreased the fecal bacteria colony-forming unit (CFU) counting. 16S sequencing also showed a significant decrease of the operational taxonomic unit (OTU) counting and relative abundance of most of the major bacteria in the feces after antibiotics treatment. Following two weeks' antibiotics feeding, the gut microbiota depletion affected the behaviorally expressed pain in the three models in modality specific manners: in CCI model, antibiotics treatment inhibited thermal hyperalgesia but not mechanical allodynia; in chemotherapy model, both thermal hyperalgesia and mechanical allodynia were prevented; and in STZ model, however, neither thermal hyperalgesia nor mechanical allodynia was altered by the antibiotics treatment. Oral gavage of fecal bacteria from SPF mice restored part of gut microbiota but fully reversed gut microbiota depletion-induced inhibition of CCI-induced thermal hyperalgesia. 16S sequencing of fecal bacteria shows that Bacterioides genus of gut microbiota had a strong correlation with the painful behavior. The relative abundance of Bacterioides genus was increased following CCI treatment. After fecal bacteria transplantation in antibiotics-treated CCI mice, Bacterioides genus was reestablished in the gut and CCI-induced thermal hyperalgesia was rescued. In addition, cytokine array assay showed that antibiotics treatment caused significant changes in multiple cytokines in the dorsal root ganglion and spinal cord in CCI mice. This study demonstrates different roles of gut microbiota in the development of chronic pain in different forms of stress and may open up a new avenue of pain therapy by manipulating gut microbiota.

Disclosures: **P. Ma:** None. **C. Qiu:** None. **G. Wu:** None. **R. Mo:** None. **Y. Zhao:** None. **X. Song:** None.

Poster

220. Preclinical and Clinical Studies in Peripheral Nerve Injury and Neuropathic Pain

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 220.25/I41

Topic: D.03. Somatosensation – Pain

Support: R01DK117404 - NIDDK
CVRI Collaborative Grant, Loyola University Chicago

Title: Gut bacteria metabolites as novel therapeutic strategies for obesity-related neuropathy

Authors: *R. BONOMO[1], T. COOK[1], C. GAVINI[1], L. GAUTRON[2], B. LAYDEN[3], V. AUBERT[1];
[1]Loyola Univ. Chicago, Maywood, IL; [2]Univ. of Texas Southwestern, Dallas, TX; [3]Univ. of Illinois Chicago, Chicago, IL

Abstract: Obesity is considered a 21st century epidemic, affecting over 2 billion people worldwide. This condition is accompanied by complications, including peripheral neuropathy (PN). PN is a highly prevalent disease, characterized by a distal to proximal nerve degeneration with current no available treatment. The initial stages of PN are clinically presented as peripheral tactile allodynia and thermal hyperalgesia, that later progress to loss of sensation. Despite high prevalence, the molecular mechanisms underlying the disease onset are poorly understood and affected patients have no choice other than to use pain killers. Using sensory neurons-specific transgenic mice, we observed expression of short-chain fatty acid (SCFA) receptors at the surface of sensory neurons proven to be involved in painful neuropathy.
Since SCFA are metabolites secreted by gut bacteria, this data potentially links the microbiome with obesity-induced pain. Numerous studies have demonstrated that gut microbiome may play a role in the development of obesity and its associated comorbidities. Moreover, fecal microbiome transplantation (FMT) has been used to treat these metabolic disorders. Still, the relationship between gut microbiome and PN onset and progression has yet to be explored. To investigate whether gut microbiome is involved in PN in obesity, we utilized an obesity-induced allodynia mouse model developed in our lab. Our data showed that both, WD-fed mice subjected to FMT and WD-fed mice treated with SCFA, exhibited improved mechanical sensitivity when compared to WD alone and respective control groups.
In addition, we evaluated changes in i) gut bacteria composition, ii) plasma short-chain fatty acids levels, iii) gene expression in dorsal root ganglia (DRG) and sciatic nerve (SN), iv) macrophage infiltration in DRG and SN, and v) protein acetylation levels in macrophages and sensory neurons. In summary, the data presented shows that transplantation of gut bacteria from lean mice to WD-fed mice, as well as SCFA treatment, improve PN symptoms in obese mice. Our data also suggest that the gut microbiome metabolites may change sensory neurons function

via modulating short chain fatty acid receptor located at the surface of sensory neurons and macrophages. The gut microbiome, its metabolites and short chain fatty acid receptors could be novel valuable targets to study the delay or cure of debilitating pain associated with obesity, providing alternative therapeutic targets to replace the use of addictive pain killers.

Disclosures: R. Bonomo: None. **T. Cook:** None. **C. Gavini:** None. **L. Gautron:** None. **B. Layden:** None. **V. Aubert:** None.

Poster

220. Preclinical and Clinical Studies in Peripheral Nerve Injury and Neuropathic Pain

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 220.26/I42

Topic: D.03. Somatosensation – Pain

Support: NIH Grant R01NS103812

Title: Ectopic activity from injured dorsal root ganglion neurons triggers hyperalgesia and allodynia in rats with spinal nerve ligation

Authors: *B. PAN, D. CHAO, G. YU, Q. HOGAN;
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Abstract: Peripheral nerve injury may cause hyperalgesia, allodynia and ongoing pain by increasing the ectopic activity of primary afferent neurons. There is still uncertainty whether injured afferents or their uninjured neighbors are the main source that contributes to pain behavior after nerve injury. To address this, we used the spinal nerve ligation (SNL) rat model, in which almost all L5 afferent fibers are injured (axotomy), while almost all 4th lumbar (L4) fibers remain uninjured, although they share distal fascicles with degenerating distal segments of L5 axotomized neurons. To selectively block ascending impulses separately in L4 and L5, we used dorsal root ganglion (DRG) field stimulation (GFS), which blocks the afferent passage to the dorsal root of activity originating both in the sensory neuron somata in the DRG, and in the sensory neuron peripheral process (receptive fields and injury sites). After SNL, *in vivo* single unit recordings from fibers teased from the L5 dorsal root showed higher spontaneous activity (56% of fibers) than L4 (21%) after SNL. GFS blocked this spontaneous activity. GFS delivered at the L5 DRG blocked behavioral hyperalgesia (pin), allodynia (von Frey), and ongoing pain (conditioned-place preference), whereas GFS at L4 did not. Finally, *in vivo* single unit dorsal horn (DH) recording of wide dynamic range (WDR) neurons showed that following SNL, L5 GFS inhibited spontaneous activity, and reversed the depressed mechanical stimulus threshold to induce action potential firing of DH neurons during punctate mechanical stimulation (both 26g/1.1mm tip, and 16g/0.1mm tip), while L4 GFS lacked these effects. Our results suggest that injured neurons, not uninjured neighbors, trigger hyperalgesia, allodynia, and ongoing pain by

axotomy-induced spontaneous firing of DRG neurons, which facilitates firing of dorsal horn projection neurons.

Disclosures: **B. Pan:** None. **D. Chao:** None. **G. Yu:** None. **Q. Hogan:** None.

Poster

220. Preclinical and Clinical Studies in Peripheral Nerve Injury and Neuropathic Pain

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 220.27/I43

Topic: D.03. Somatosensation – Pain

Support: P20GM103643
R01EY026145

Title: The contribution of Sox11 expression in Nav1.8-positive neurons to nerve regeneration after acute and chronic corneal injury

Authors: *C. SULLIVAN[1,3], S. LEE[1,4], I. MENG[1,2,3];
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Abstract: *Sox11* expression is observed in primary afferent neurons early on after peripheral nerve injury and appears to play a critical role in regeneration [1,2]. Currently, little is known about the role of *Sox11* in nerve regeneration following corneal injury. The aim of this study was to examine corneal nerve regeneration in two corneal injury models, lacrimal gland excision (LGE) and corneal abrasion (CA), using Nav1.8Cre-*Sox11*-tdTomato (Sox11 KO) and control mice. Lacrimal gland excision-induced dry eye reduces the aqueous component of tears, resulting in persistent corneal epithelial cell damage and retraction of corneal afferent nerve terminals. Corneal abrasion (CA) mechanically removes the corneal epithelium and axon terminals, which are then allowed to recover. Corneal fluorescein was used to examine the severity of epithelial damage, mechanical sensitivity was evaluated using a corneal aesthesiometer and nerve terminal density was imaged from whole mounts using a Keyence BZ-X700 fluorescent microscope and analyzed with FIJI. We found that while corneal innervation density decreases between 1-2 weeks following LGE in control animals, nerves can regenerate to near normal levels by 4 weeks, albeit in a disorganized manner. In *Sox11* KO animals, innervation density was greatly reduced at the 4-week time point when compared to control animals. Corneal epithelial cell damage, as determined with fluorescein staining, was similar between Sox11 KO and control animals over the 4 -weeks after LGE. Directly following CA-induced injury, both control and Sox11 KO animals showed significant decreases in innervation density at 24 hours. By 48 hours after injury, Sox11 KO animals showed a small yet significant

increase in nerve growth. Both control and Sox11 KO animals demonstrated comparable reductions in corneal epithelial cell damage 48 hours after injury. Additionally, Sox11 KO animals had lower mechanical thresholds when compared to control animals. Taken together, these results provide support for a role of *Sox11* in nerve regeneration and hypersensitivity following injury.

References:

[1] Jankowski MP, McIlwrath SL, Jing X, et al. Sox11 transcription factor modulates peripheral nerve regeneration in adult mice. *Brain Res*. 2008;1256:43-54. doi:10.1016/j.brainres.2008.12.032)

[2] Li Y, Struebing FL, Wang J, King R, Geisert EE. Different Effect of *Sox11* in Retinal Ganglion Cells Survival and Axon Regeneration. *Front Genet*. 2018;9:633. Published 2018 Dec 18. doi:10.3389/fgene.2018.00633

Disclosures: C. Sullivan: None. **S. Lee:** None. **I. Meng:** None.

Poster

220. Preclinical and Clinical Studies in Peripheral Nerve Injury and Neuropathic Pain

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 220.28/I44

Topic: D.03. Somatosensation – Pain

Title: Riboregulation of the essential translation initiation scaffold eIF4G as a strategy to attenuate mechanical hyperalgesia

Authors: C. ZHANG[1], ***J. DE LA PENA**[1], Q. ZHOU[1], T. SHUKLA[1], E. YETKIN[1], T.-F. LOU[1], F. MORCOS[1], Z. CAMPBELL[2];
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Abstract: Noxious stimuli trigger rapid induction of nascent translation in nociceptors. All messenger RNA possess a conserved M7G cap structure. In the cytoplasm, the cap interacts with a tripartite protein complex called eIF4F. The transient association of the complex with the cap controls which mRNAs are selected for translation and thus serves as a critical regulatory checkpoint. The vast majority of inhibitors of the complex target the cap-binding subunit, eIF4E. We sought to determine if the complex bound better to specific RNA sequences near the cap and if such a bias confers differences in translational efficiency. Seven protein complexes containing subunits of eIF4F and associated factors were subjected to unbiased comprehensive analysis using RNA selection and high throughput sequencing. We found a single highly structured RNA element, termed the eIF4G Complex Disrupting RNA decoy or FORAY that preferentially bound eIF4F. We mapped the interaction to the 175 kilo Dalton scaffolding protein eIF4G. Insertion of the FORAY into the 5' untranslated region of a reporter confers modest IRES activity. We generated chemically stabilized forms of the FORAY that bind with high affinity

and specificity to eIF4G. The FORAY diminishes nascent translation of a reporter gene by ~80%. To probe the underlying mechanism, we examined binding of 4G to protein partners in the presence of the FORAY and characterize loss of specific protein partners known to stimulate initiation. We examined the role of eIF4G in pain sensitization using hyperalgesic priming models. We propose that riboregulation of eIF4G provides a novel means to transiently inhibit cap-depdendent translation and as a means to attenuate pain amplification behaviors.

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Poster

220. Preclinical and Clinical Studies in Peripheral Nerve Injury and Neuropathic Pain

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 220.29/J1

Topic: D.03. Somatosensation – Pain

Support: DFG Ri817/13-1
Funds from the University of Wuerzburg
Chinese Scolarship Council

Title: Mechanical hypersensitivity through opening of the myelin barrier via tight junction protein knockdown

Authors: J. T. S. CHEN[1], X. HU[3], I. OTTO[4], R. BLUM[2], I. BLASIG[5], ***H. L. RITTNER**[3]; [1]Anesthesiol., [2]Inst. Clin. Neurobio., Univ. Hosp. Wuerzburg, Wuerzburg, Germany; [3]Anesthesiol., Univ. Hosp. of Wuerzburg, Wuerzburg, Germany; [4]Anestesiology, Universtity Hosp. of Wuerzburg, Wuerzburg, Germany; [5]Leibnitz Forschungsinstitut Mol Pharmakologie, Belrin, Germany

Abstract: Background: Peripheral nerves are sealed by three barriers: the blood-nerve barrier (BNB) consisting of the perineurium surrounding nerve fascicles and the endothelium of endoneurial blood vessels and the myelin barrier (MB) formed by Schwann cells. The MB situated in the paranodal region and in the mesaxon. Barriers are sealed by tight junction proteins (TJPs) like claudins or occludin. BNB tightness is ensured by claudin (cldn)-1, ZO-1 and JAM-C expressed in the perineurium and cldn-5 in endoneurial vessels. In neuropathy like in chronic constriction injury (CCI), the BNB is opened leading to diffusion of possible toxic metabolites and immune cell invasion. Here, we explored the MB tightness and their sealing TJPs in neuropathy focusing on cldn-19 and -12. Cldn-19 is found in the PNS, the kidney and the retinal pigment epithelium. Cldn-12 is known to be expressed in intestinal epithelia and the BBB, but only distantly related to other TJPs. Methods: Animal experiments were approved by local authorities. Neuropathy was induced in male mice via CCI of the sciatic nerve. Thermal and

mechanical nociceptive thresholds as well as tight junction protein expression and barrier properties were analyzed in WT as well as cldn-12 KO mice and after CCI. Results were validated by siRNA mediated knockdown applied locally at the sciatic nerve. Results: In naïve mice, cldn-12 was expressed in S100-positive Schwann cells, cldn-19 mainly in the paranode. CCI caused a leakiness of the BNB and the MN for large and small molecules, Evan blue albumin (68 kD) and sodium fluorescein (340 Da). This was accompanied by in a downregulation of cldn-1, -5, -12 and -19 mRNA and immunoreactivity. SiRNA-mediated knockdown of cldn-12 and -19, but not cldn-5 increased MB leakiness and elicited acute mechanical hypersensitivity. Male cldn-12 KO mice displayed not gross neurological abnormalities but were more sensitive to mechanical stimuli. Male but not female naive cldn-12 KO mice were characterized by a loss of small fibers and an increased permeability of the perineurium and the MB. Tightness of the endoneurial vessels, the intestinal or blood brain barrier were unaffected. While cldn-12 mRNA and protein were completely lost, other TJP were also reduced like cldn-1 and cldn-19 mRNA and protein in male cldn-12 KO mice. Conclusion: Cldn-12 regulates MB and perineurial tightness sex-dependently and affects the expression of other TJPs pointing towards a regulatory role of this TJP. MB leakiness renders mice more sensitive to mechanical stimuli. Proper barrier sealing of Ad nociceptive fibers is essential for normal nociceptive thresholds.

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Poster

220. Preclinical and Clinical Studies in Peripheral Nerve Injury and Neuropathic Pain

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 220.30/J2

Topic: D.03. Somatosensation – Pain

Title: Transcriptome analysis of the immune and neuroinflammatory responses associated with peripheral nerve injury

Authors: *A. KRASOWSKA-ZOLADEK[1], D. LOVATT[1], R. SANOJA[1], K. TANIS[2], M. PEARSON[1], A. TAMBURINO[2], L. LI[3], K. ZHANG[3], V. PETERSON[3], X. WANG[1], J. USLANER[1];
[1]Neurosci., Merck&Co., West Point, PA; [2]Genet. and Genomic, Merck&Co., Inc, West Point, PA; [3]Genet. and Genomic, Merck&Co., Inc, Boston, MA

Abstract: Damage to nociceptors that relay pain information from the periphery to the spinal cord can result in neuropathic pain, which is characterized by hyperalgesia, allodynia, and spontaneous pain. Accumulated evidence suggests that one of the factors that contributes to neuropathic pain is the infiltration of immune cells in response to nerve injury, leading to

production and secretion of various immune mediators such as cytokines, chemokines, and growth factors. These mediators promote neuroimmune activation, sensitize nociceptors, and contribute to pain hypersensitivity. In turn, nociceptor neurons release neuropeptides and neurotransmitters that regulate innate and adaptive immune responses. To further elucidate the immune and neuroinflammatory changes post nerve injury, we conducted a series of transcriptome analysis using both bulk and single-cell RNAseq of neuroma tissue as well as contralateral sciatic nerve over a period of 60 days post spared nerve injury (SNI) in rodents. The transcriptome analysis revealed a robust infiltration of immune cells post nerve injury, including mast cells, M1 and M2 macrophages, and T-cells in a time-dependent manner. RNAscope analysis further confirmed the infiltrated immune cells are within the injured nerve tissue. Associated with the immune cell infiltration, cytokines, chemokines and the receptors for these immune mediators and neurotransmitters are also differentially regulated post injury. A critical question for the future is to establish to what extent this immune response contributes to neuropathic pain in human diseased conditions. This work is important for understanding immune modulation of neuropathic pain and could potentially lead to the identification of novel therapeutic targets.

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Poster

221. Touch: Thalamic-Cortical Processing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 221.01/J3

Topic: D.04. Somatosensation – Touch

Support: NSERC PGSD3-519263-2018
Canada First Research Excellence Fund
BrainsCAN

Title: Integration of tactile information from multiple fingers in human primary sensory cortex measured using high-resolution fMRI

Authors: *S. ARBUCKLE[1], A. PRUSZYNSKI[2], J. DIEDRICHSEN[3];
[1]Brain and Mind Inst., [2]Brain and Mind Institute, Physiol. and Pharmacology, Psychology, [3]Brain and Mind Institute, Computer Science, Statistics and Actuarial Sci., Univ. of Western Ontario, London, ON, Canada

Abstract: Processing tactile information from the finger tips, be it cutaneous information from the glabrous skin, or proprioceptive information conveying the spatial arrangement of the

fingers, is central to the ability to skillfully manipulate objects. Sensory information across fingers must be integrated to yield a percept of the object being manipulated. Here we investigated the integration of tactile information across multiple fingers in human primary sensory cortex (S1). Neurons in all subregions of S1, Brodman areas (BA) 3a, 3b, 1, and 2, are responsive to finger stimulation. Direct sensory input arrives in BA 3a and 3b in an orderly finger-specific fashion. These areas then project to BA 1 and 2, which are hypothesized to integrate information from multiple fingers. To study this sensory integration in humans, we used high resolution functional magnetic resonance imaging (fMRI, 7T, 1.4mm isotropic, TR=1.5s) to measure activity patterns evoked in left S1 during passive single- and multi-finger stimulation of the right hand. The glabrous skin of each fingertip was independently indented with a small brass rod (radius = 1mm, force ~3 Newtons).
We found significant finger encoding in all subregions of S1. Finger encoding and overall activity was weakest in BA 3a, likely because this area receives predominantly proprioceptive input. In BA 3b and 1, clear encoding for individual fingers was found, and activity increased with the number of stimulated fingers. Average activity also increased with the number of stimulated fingers in BA 2, but the finger encoding was weaker, suggesting larger overlap between finger representations.
We then investigated how information from multiple fingers was integrated. To test this, we used pattern component modelling (Diedrichsen et al., 2017, NeuroImage). We fit a linear integration model where multi-finger activity patterns were predicted as the sum of the constituent single finger patterns. To account for local inhibition, each pattern was scaled by a constant dependent on the number of fingers stimulated. This linear-nonlinear model could account for the representations in all subregions of S1 as good as a noise-ceiling model. In conclusion, integration of single finger information in S1 appears to be linear (at the resolution of fMRI) in a passive paradigm in which the fingers are independently stimulated.

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Poster

221. Touch: Thalamic-Cortical Processing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 221.02/J4

Topic: D.04. Somatosensation – Touch

Support: JSPS KAKENHI Grant Number JP18K17725

Title: Afferent pathway to the ipsilateral somatosensory cortex in human

Authors: *D. ISHII[1,4], K. ISHIBASHI[5], Y. KAKU[2], H. YUINE[3], S. YAMAMOTO[2], A. YOZU[1], Y. KOHNO[1];
[1]Ibaraki Prefectural Univ. of Hlth. Sci., Ami, Japan; [2]Ibaraki Prefectural Univ. of Hlth. Sci.,

Ibaraki, Japan; [3]Occup. Therapy, Sch. of Healthcare, Ibaraki Prefectural Univ. of Hlth. Sci., Ami, Japan; [4]Cognitive Behavioral Physiol., Chiba Univ. Grad. Sch. of Med., Chiba, Japan; [5]Ibaraki Prefectural Univ. of Hlth. Sci. Hosp., Ami, Japan

Abstract: Damage to the sensorimotor pathway following stroke causes various functional disorders, such as motor and sensory impairments. Several studies have revealed that neural plasticity compensates for the loss of motor function after stroke. Sensory impairment is less likely to recover than motor impairment. We hypothesize that one reason for this difference is that the sensory pathway is less diverse than the motor pathway, and thus a compensatory route is less likely to occur. Here, we investigated the afferent pathway from sensory neurons to the ipsilateral somatosensory cortex in human using the paired median nerve somatosensory evoked potentials (SEP). Paired median nerve SEPs were recorded at CP4 with a reference of Fz in the International 10-20 System. In addition, we recorded the nerve action potentials at the ipsilateral Erb's point. Paired median nerve stimulation with varying interstimulus intervals (ISI; 1, 2, 3, 5, 10, 20, 40, 60, and 100 ms) was performed to test the influence of the first stimulus on the N20 and P25 components induced by the second stimulus. The second stimulation resulted in a reduction in the N20 and N20/P25 amplitude when the ISI was 10 ms, whereas no changes were observed for other ISI conditions. These results revealed that the somatosensory pathway has less diversity.

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Poster

221. Touch: Thalamic-Cortical Processing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 221.03/J5

Topic: D.04. Somatosensation – Touch

Support: Sasakawa Scientific Research Grant from The Japan Science Society
JSPS Kakenhi (Grant number JP18H03134)

Title: The effect of acute aerobic pedaling exercise on the inhibitory pathway in the primary somatosensory cortex and somatosensory function

Authors: *Y. YAMAZAKI, K. YAMASHIRO, H. ONISHI, N. OTSURU, S. KOJIMA, K. SAITO, D. SATO;
Niigata Univ. of Hlth. and Welfare, Niigata, Japan

Abstract: Acute aerobic exercise has a beneficial effect on the brain function. However, it remains unclear whether acute aerobic exercise modulates the activity and function of the

primary somatosensory cortex (S1). Therefore, we aimed to explore the effect of acute aerobic exercise on the S1 inhibitory pathway and somatosensory function. Fifteen healthy, right-handed participants carried out two experiments. In experiment 1, somatosensory evoked potentials (SEP) and paired pulse inhibition (PPI) were measured. Interstimulus interval (ISI) of PPI was set at 5, 30, and 100 ms (PPI_5ms, PPI_30ms, PPI_100ms). PPI was defined as the ratio between the amplitude of the second component of paired pulse SEP and the amplitude of the single SEP component. In experiment 2, somatosensory temporal discrimination task was used to assess the somatosensory function. Single or paired stimuli (10 - 120 ms ISI) were delivered in random order, and participants responded by pushing a button to denote whether the number of stimuli was one or two. We calculated 50%- and 75%-threshold, and reaction time (RT). In both experiments, participants performed in three conditions: 20 min of moderate intensity exercise (Mod Ex), 30 min of low intensity exercise (Low Ex), and 30 min of seated rest (CON), on different days. SEP and temporal discrimination task were measured before and after intervention (5, 20, 40, and 60 min). In experiment 1, single SEP (N20, P27, and N20-P27) did not change in any of the conditions. On the other hand, PPI_30ms significantly decreased at 20 min after Mod Ex ($p < 0.05$). In experiment 2, 50%- and 75%-threshold, and RT did not change in any of the conditions. The changes in PPI_30ms at 5 min after Low Ex significantly correlated with the changes in 50%-threshold ($r = -0.609$, $p = 0.027$), and changes in PPI_5ms at 40 min after Low Ex significantly correlated with the change in 50%- and 75%-threshold (50%: $r = -0.715$, $p = 0.006$; 75%: $r = -0.793$, $p = 0.001$). One of the explanations for the decrease of PPI after Mod Ex is the secretion of neuromodulators, such as noradrenaline and serotonin. In contrast to the changes in PPI, acute aerobic exercise did not improve temporal discrimination irrespective of exercise intensity. The lack of change in temporal discrimination might be due to the individual variability of the modulation of S1 inhibitory pathway induced by acute aerobic exercise. In conclusion, acute aerobic pedaling exercise modulates the excitability of the S1 inhibitory pathway, but it does not improve temporal discrimination. On the other hand, the degree of change in temporal discrimination was associated with modulation of the S1 inhibitory pathway by acute aerobic exercise.

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Poster

221. Touch: Thalamic-Cortical Processing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 221.04/J6

Topic: D.04. Somatosensation – Touch

Support: NRF of Korea Grant 2016M3C7A1904984

Title: Dorsal and ventral streams of somatosensory processing - A combined DCS and high-gamma band mapping study

Authors: ***S. RYUN**[1], M. KIM[1], J. KIM[3], C. CHUNG[2];
[2]Neurosurg., [1]Seoul Natl. Univ., Seoul, Korea, Republic of; [3]Seoul Nat Univ., Seoul, Korea, Republic of

Abstract: Human somatosensory processing for perception involves complex co-operation among multiple brain areas. Although its exact mechanism has been poorly understood, it is considered that somatosensory perception is largely affected by specific neuronal activation of many cortical regions beyond the primary somatosensory cortex (S1) via top-down or bottom-up streams. However, many previous studies have focused on the intervention for the early somatosensory stage, the S1, to elicit or investigate specific somatosensory perception. The present study combines direct cortical stimulation (DCS) data for eliciting somatosensation and high-gamma band (50 to 150 Hz) mapping data during tactile stimulation. To do this, we generated normalized functional maps of the elicited somatosensation by DCS on subdural ECoG electrodes from nearly 50 patients with intractable epilepsy. Additionally, we constructed four-dimensional maps of ECoG high-gamma activities during various sensorimotor tasks including hand grasping, elbow flection, vibrotactile and texture stimulation from 20 (for movement tasks) and 30 (for tactile tasks) epilepsy patients. We found that the artificial somatosensory perception is elicited not only from conventional somatosensory-related areas such as the primary and secondary somatosensory cortices, but also from widespread network including inferior parietal lobule (IPL), premotor cortex (PM) and posterior parietal cortex. Interestingly, the distributions of electrode locations elicited somatosensation showed distinct spatial differences depending on the quality of somatosensation. Namely, the DCS on the dorsal part of the parietal area often induced action-related somatosensation such as proprioception, whereas that on the ventral part of fronto-parietal area generally elicited tactile sensation. Furthermore, our 4-D high-gamma mapping results of movement and passive tactile stimulation tasks indicate considerable similarity in spatial distribution between high-gamma and DCS functional maps. These findings provide evidence for the dorsal and ventral streams of somatosensory system.

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Poster

221. Touch: Thalamic-Cortical Processing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 221.05/J7

Topic: D.04. Somatosensation – Touch

Support: EU H2020 FET project 829168 ph-coding

Swedish Research Council Project Grant #2016-01656
Hjärnfonden

Title: Focal stroke affects tactile processing in neocortical neurons at large distances from the lesion

Authors: *A. WAHLBOM, J. M. D. ENANDER, F. BENGTSSON, H. JÖRNTELL;
Dept. of Exptl. Med. Sci., Neural Basis of Sensorimotor Control, Lund, Sweden

Abstract: Viewed through the conceptual filter of a functionally parceled neocortex, there are multiple clinical observations after localized neocortical strokes that seem paradoxical in that they have widespread functional deficits. Here we provide an in-depth analysis of the changes in the function of the neocortical neuronal networks after distant focal stroke-like lesions in the ketamine-anesthetized rat. Using a recently introduced high resolution analysis of neuronal information processing, consisting of pre-set spatiotemporal patterns of tactile afferent activation against which the neuronal decoding performance can be quantified, we found that stroke-like lesions in distant parts of the cortex significantly degraded the decoding performance of individual neocortical neurons in the primary somatosensory cortex. This degrading effect was not due to changes in the firing frequency of the neuron. The degrading effect was stronger the higher the decoding performance of the neuron, which indicates a specific impact on the information processing capacity in the cortex. These findings suggest that even primary sensory processing depends on widely distributed cortical networks and could explain observations of focal stroke lesions affecting a large range of functions.

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Poster

221. Touch: Thalamic-Cortical Processing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 221.06/J8

Topic: D.04. Somatosensation – Touch

Support: NIH EY014681
ONR N000014-14-0670
Brown Center for Vision Research
Brown OVPR Seed

Title: Macaques as a model for haptic object recognition

Authors: *R. L. MILLER, D. L. SHEINBERG;
Neurosci., Brown Univ., Providence, RI

Abstract: Although humans are clearly adept at recognizing objects by touch, very little is known about how this is accomplished at a single neuron level. One reason for this is likely the relative complexity of presenting physical objects to animals in a lab setting while having the ability to record brain activity. We've developed a new automated system capable of presenting any of a large set of physical objects to a non-human primate for inspection on a per-trial basis while simultaneously allowing us to monitor precisely when and where the object is being touched. Further, we've coupled high-speed video of a monkey's reaching movements with a neural-network classifier that allows us to track individual joints and thereby characterize the grasp shape at any given moment. With this system, we've found that monkeys can quickly learn novel shapes by touch alone, showing significant learning in as few as 20 presentations. Once learned, they are also quite quick to decide whether the object being felt is a match to a visual stimulus, requiring around 350 ms haptic exploration on average. They also appear to employ similar strategies as humans, looking at the same part of an object (presented on a screen) that they're touching. Finally, we've identified task-related activity in single neurons in motor, somatosensory, and STS cortices which demonstrate differential firing based on shape. This demonstrates that macaques can provide a viable model for studying haptic object recognition of novel, complex objects in a setting that allows for single-unit recording.

Disclosures: R.L. Miller: None. **D.L. Sheinberg:** None.

Poster

221. Touch: Thalamic-Cortical Processing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 221.07/J9

Topic: D.04. Somatosensation – Touch

Support: NIH T32 NS007222
NSF Graduate Research Fellowship Program
NIH Grant R01GM111293
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A. Alfred Taubman Medical Research Institute

Title: Neuronal evidence for adaptive reconfiguration during subanesthetic doses of inahled nitrous oxide

Authors: *M. WILLSEY[1], C. S. NU[2], S. R. NASON[2], K. E. SCHROEDER[5], B. HUTCHISON[2], E. J. WELLE[2], P. G. PATIL[1], C. A. CHESTEK[3], G. A. MASHOUR[4]; [1]Neurosurg., [3]Biomed. Engin., [4]Anesthesiol., [2]Univ. of Michigan, Ann Arbor, MI; [5]Neurosci., Columbia Univ., New York, NY

Abstract: It has been suggested that the brain undergoes adaptive reconfiguration in order to optimize network organization in response to a functional perturbation. Large-scale network studies in rodents and humans have investigated this process after exposure to general anesthetics. To assess adaptive reconfiguration at the neuronal level after a pharmacologic challenge, we administered subanesthetic doses nitrous oxide (N_2O) and studied the dynamical changes of spiking rate, spectral content, and somatosensory information transfer to motor cortex. Two Rhesus macaques were implanted with Utah microelectrode arrays in the hand area of motor cortex. During an increase from 0% to 70% inhaled N_2O in oxygen, the evolution of spiking rate and spectral content was measured. To evaluate the strength of somatosensory information transfer from primary sensory (S1) to primary motor (M1) cortex, fingers were lightly brushed using a cotton tip and a naïve Bayes classifier based on neural response was used to classify which finger was brushed at "early" (15-35 min after beginning N_2O) and "late" (45 - 65 min) time points. In M1 neurons, we found that average multi-unit spiking rate increased by 4.2 ± 0.52 Hz to 17 Hz in Monkey W ($p < 0.001$) and by 1.27 ± 0.55 Hz to 9.4 Hz in Monkey N ($p = 0.02$). Power spectral densities also showed a robust increase in beta and gamma band power with subanesthetic N_2O administration. Of relevance to adaptive reconfiguration, we found that S1->M1 information transfer was initially disrupted as evidenced by the inability to classify the finger brushed during early exposure. However, this information transfer rebounded in the later stage of exposure, even with continued N_2O administration, and allows improved finger classification from 33% (chance, based on three possible fingers) to 57% in Monkey W and from 20% to 67% in Monkey N. Finally, the normalized modulation depth, a measure of neuron specificity to the brushed finger, increased from 0.20 to 0.31 in Monkey W ($p = 0.040$) and from 0.23 to 0.34 in Monkey N ($p = 0.046$) from early to late stages of N_2O administration. The rebound of somatosensory information in motor cortex represents direct evidence at the neuronal level for adaptive reconfiguration of functional networks.

Disclosures: **M. Willsey:** None. **C.S. Nu:** None. **S.R. Nason:** None. **K.E. Schroeder:** None. **B. Hutchison:** None. **P.G. Patil:** None. **C.A. Chestek:** None. **G.A. Mashour:** None. **E.J. Welle:** None.

Poster

221. Touch: Thalamic-Cortical Processing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 221.08/J10

Topic: D.04. Somatosensation – Touch

Support: Human Brain Project - 785907 HBP SGA2
MINECO/FEDER - BFU2017-88549
MINECO/FEDER - BFU2012-36107

Title: Posterior thalamic nucleus axon terminals have different structure and functional impact in the motor and somatosensory vibrissal cortices

Authors: *D. CASAS TORREMOCHA[1], C. PORRERO[1], J. RODRÍGUEZ-MORENO[1], M. GARCÍA-AMADO[1], J. H. LUBKE[2], Á. NÚÑEZ[1], F. CLASCÁ[1];
[1]Anat. and Neurosci., Autónoma De Madrid Univ., Madrid, Spain; [2]Res. Ctr. Juelich, Juelich, Germany

Abstract: Rodents extract information about nearby objects from the movement of their whiskers through dynamic computations that are carried out by a network of forebrain structures that includes the thalamus and the primary sensory (S1BF) and motor (M1wk) whisker cortices. The posterior nucleus (Po), a higher order thalamic nucleus, is a key hub of this network, receiving cortical and brainstem sensory inputs and innervating both motor and sensory whisker-related cortical areas. In a recent study in rats, we showed that Po inputs differently impact sensory processing in S1BF and M1wk. Here, in C57BL/6 mice, we measured Po synaptic bouton layer distribution and size, compared cortical unit response latencies to "in vivo" Po activation, and pharmacologically examined the glutamatergic receptor mechanisms involved. We found that, in S1BF, a large majority (56%) of Po axon varicosities are located in layer (L)5a and only 12% in L2-L4, whereas in M1wk this proportion is inverted to 18% and 55%, respectively. Light and electron microscopic measurements showed that Po synaptic boutons in M1wk layers 3-4 are significantly larger (~ 50%) than those in S1BF L5a. Electrical Po stimulation elicits different area-specific response patterns. In S1BF, responses show weak or no facilitation, and involve both ionotropic and metabotropic glutamate receptors, whereas in M1wk, unit responses exhibit facilitation to repetitive stimulation and involve ionotropic NMDA glutamate receptors. Because of the different laminar distribution of axon terminals, synaptic bouton size and receptor mechanisms, the impact of Po signals on M1wk and S1BF, although simultaneous, is likely to be markedly different.

Disclosures: D. Casas Torremocha: None. **C. Porrero:** None. **J. Rodríguez-Moreno:** None. **M. García-Amado:** None. **J.H. Lubke:** None. **Á. Núñez:** None. **F. Clascá:** None.

Poster

221. Touch: Thalamic-Cortical Processing

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Topic: D.04. Somatosensation – Touch

Support: European Union's Horizon 2020 (Grant Agreement no. 785907 HBP SGA2)
Ministerio de Economía y Competitividad/Fondo Europeo para el Desarrollo Regional (MINECO/FEDER) Grant BFU2017-88549

Title: The functional logic of higher-order thalamocortical axons branching to innervate multiple areas

Authors: *C. PORRERO[1], M. RUBIO-TEVES[1], D. CASAS-TORREMOCHA[1], J. RODRIGUEZ-MORENO[1], M. GARCIA-AMADO[1], M. C. BALLESTEROS-BRIONES[2], C. SMERDOU[2], T. FURUTA[3], F. CLASCA[1];
[1]Anat. and Neurosci., Autonoma de Madrid Univ., Madrid, Spain; [2]Div. of Gene Therapy and Regulation of Gene Expression, CIMA, Univ. de Navarra and Idisna, Pamplona, Spain; [3]Dept. of Oral Anat. and Neurobio., Osaka Univ., Osaka, Japan

Abstract: Thalamocortical axons from sensory relay nuclei neurons innervate the middle layers of their target cortical area heavily and in a spatially focused manner. Axons arising from neurons in different portions of these nuclei are overall organized in point-to point fashion ad thus create isomorphic representation of the corresponding sensory receptor sheets in a primary area of the cerebral cortex. In contrast, “higher-order” (HO) thalamic nuclei axons send back to cortex signals received mainly from the cortex itself, and usually branch of innervate several separate cortical areas and often the striatum as well. The logic of HO branched wiring remains poorly understood. Here, we show that the axons from the Posterior nucleus (Po), a HO nucleus of the mouse thalamus, systematically branch to target several cortical (and often also striatal) domains that process sensory/motor information about the same body part. Single-cell and micropopulation tracing data reveal that Po neurons innervating domains related to the same body part are clustered together; as a result, a continuous “connectivity map” links each point of Po with multiple cortical areas, in somatotopic fashion. Moreover, Po axon varicosities (putative synaptic boutons) vary markedly in laminar distribution, and often in size, between the various target areas. These observations reveal that higher-order neuron axons may modulate functional connectivity among cortical and striatal neuronal populations involved in movement/sensation of specific body regions.

Disclosures: C. Porrero: None. **M. Rubio-teves:** None. **D. Casas-torremocha:** None. **J. Rodriguez-moreno:** None. **M. Garcia-amado:** None. **M.C. Ballesteros-briones:** None. **C. Smerdou:** None. **T. Furuta:** None. **F. Clasca:** None.

Poster

221. Touch: Thalamic-Cortical Processing

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Program #/Poster #: 221.10/J12

Topic: D.04. Somatosensation – Touch

Support: R01NS094184
R01EY022338
5F31EY028812-02

Title: Mouse S1 and V1 each initiates a feedback circuit to itself via higher-order thalamus

Authors: *A. J. MILLER, S. M. SHERMAN;
Neurobio., Univ. of Chicago, Chicago, IL

Abstract: Higher-order (HO) thalamic nuclei are important contributors to early sensory processing, and transthalamic feedforward pathways connecting cortical areas through HO thalamus have been recognized. However, HO thalamic nuclei POm and Pul also innervate primary sensory cortices S1 and V1, respectively, and these projections are not well understood. Both POm and Pul have heterogeneous inputs and outputs, clouding our understanding of the different projection populations. We aimed to identify the source of driving input to these HO thalamic projections to S1 and V1 and then determine the synaptic properties of the identified connections by using an intersectional anatomical and physiological approach to map and characterize the synaptic inputs and outputs of POm and Pul in mice. Sub-population specific viral strategies were used to deliver fluorescent reporters and retrograde labeling machinery (pseudotyped ΔG Rabies) to the POm → S1 pathway, labeling its presynaptic inputs while ignoring other projections to POm. Additionally, virally mediated electron microscopy allowed for ultrastructural analysis of synapse morphology, and *in vitro* optogenetics experiments targeting retrolabeled POm → S1 cells to determine the physiological properties, especially the driver/modulator identity, of the synaptic inputs.
These experiments have identified and characterized a novel transthalamic *feedback* circuit, wherein layer 5 projections from S1 itself drive the POm population projecting back to S1. When combined with previous work from our group establishing that glutamatergic POm projections ultimately *modulate*, rather than *drive*, activity in S1, we conclude that S1 modulates its own activity via POm.
Our preliminary data and ongoing work, along with existing anatomical literature, suggest that the same transthalamic feedback loop exists between V1 and Pul, which may have similar physiological properties. Therefore, this recurrent loop between primary sensory cortex and HO thalamus may be conserved across sensory systems and reflect a generalized pattern of thalamocortical organization.
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Disclosures: A.J. Miller: None. **S.M. Sherman:** None.

Poster

221. Touch: Thalamic-Cortical Processing

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Topic: D.04. Somatosensation – Touch

Support: European Union Seventh Framework Programme - Human Brain Project

MINECO

Title: Axons from a higher-order thalamic nucleus innervating separate cortical areas establish structurally different synapses in each area

Authors: *J. RODRIGUEZ-MORENO[1], C. PORRERO[1], A. ROLLENHAGEN[2], L. ALONSO-NANCLARES[3], R. YAKOUBI[2], A. SANTUY[3], A. MERCHAN-PEREZ[3], J. DEFELIPE[3], J. LÜBKE[2], F. CLASCA[1];
[1]Anat. and Neurosci., Autonoma de Madrid Univ., Madrid, Spain; [2]Res. Ctr. Juelich, Juelich, Germany; [3]Univ. Politecnica de Madrid, Pozuelo de Alarcon, Spain

Abstract: Rodents identify objects with their motile vibrissae through a brain network that primarily involves two thalamocortical pathways: a) the Ventral Posteromedial nucleus (VPM) axons targeting the vibrissal primary somatosensory cortex (S1BF) layer (L)4; and b) the Posterior nucleus (Po) axons targeting S1BF L5a and L1, as well as the vibrissal motor cortex (M1wk). Using high-end 3D-electron microscopy on labeled thalamocortical axons, we investigated possible structural differences between S1BF-L5a, S1BF-L1 and M1wk-L4 Po synapses. S1BF-L5a boutons are smaller in volume (-39%), mitochondrial content (-25%) and neurotransmitter vesicle pool size (-36%) than M1wk-L4 boutons. Most Po synapses target dendritic spines (83% S1BF-L5a; 96% S1BF-L1; 94% M1wk-L4). Postsynaptic density (PSD) areas are ~70% smaller in S1BF-L5a than S1BF-L1 or M1wk-L4 synapses. These differences may underlie the divergent functional effect of Po synapses on M1wk vs. S1BF cells.

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Poster

221. Touch: Thalamic-Cortical Processing

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Program #/Poster #: 221.12/J14

Topic: D.04. Somatosensation – Touch

Support: NIH RO1NS094184
NIH RO1EY022338

Title: Patterns of corticofugal layer 5 projections to thalamic and extrathalamic targets

Authors: *J. A. PRASAD, B. J. CARROLL, S. M. SHERMAN;
Neurobio., Univ. of Chicago, Chicago, IL

Abstract: Evidence exists that cortical layer 5 (L5) neurons innervate thalamus and branch to innervate other brainstem sites; these projections to thalamus involve larger terminals than those of the L6 corticothalamic projections. We sought to begin to test the generality of this pattern. We injected Cre-dependent adeno-associated virus into S1, V1, M1 and PFC of the Rbp4-cre transgenic mouse line to express a fluorescent marker selectively within L5 cells and map their corresponding subcortical terminals. L5 terminal populations in thalamus and extrathalamic targets were larger than those of L6 thalamic terminal populations. L5 terminal morphology varied with origin and target of corticofugal projections. Specifically, inputs from S1 to the posterior medial thalamic nucleus (POm) and PFC to the submedial nucleus provided the largest terminals, whereas M1 terminals in POm and V1 terminals in pulvinar were smaller. We also observed thalamic nuclei receiving L5 inputs from multiple cortical areas, including the central lateral (CL), mediodorsal (MD) and lateral dorsal (LD) nuclei, which in some cases exhibited different topography related to cortical origin. For instance, projections from S1 and V1 converged within LD, where larger S1 terminals were located ventrally, and smaller V1 terminals laterally. Such differences were not observed for terminals converging in CL or MD from S1, V1 and PFC. Terminals within extrathalamic regions (e.g., superior colliculus, periaqueductal gray, basal ganglia) were smaller than those in POm. The larger L5 terminals suggest a driver function, as opposed to the modulatory function of the smaller L6 corticothalamic terminals, and thus the thalamic nuclei targeted by L5 terminals appear to be a central element in cortico-thalamo-cortical (transthalamic) communication. Overall, these data suggest these patterns of L5 corticofugal projections are a general feature of cortical processing.

Disclosures: **J.A. Prasad:** None. **B.J. Carroll:** None. **S.M. Sherman:** None.

Poster

221. Touch: Thalamic-Cortical Processing

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Topic: D.04. Somatosensation – Touch

Support: EU H2020 FET project 829168 ph-coding
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Title: Impact on intracellular responses to tactile inputs of information resident in the neocortical network

Authors: *J. NORRLID, J. M. D. ENANDER, H. MOGENSEN, H. JÖRNTELL;
Lund Univ., Lund, Sweden

Abstract: When the brain receives external input, the interpretation of that input depends on its internal state, which is the brain's current estimation of the relationship between the self and the

external world. This estimation is basically equal to the evolution in time of the distribution of activity across all its neurons. The current internal state is hence reflected in the activity in the synaptic connections of each neuron. Between neurons, however, the impact of the current internal state could potentially vary, depending on the network position of the neuron. Here we wanted to clarify how the state-dependency of the information of external inputs could vary between neocortical neurons. We used ketamine anesthetized rats and explored the intracellular responses of neocortical neurons to single trials out of a given set of tactile input patterns. Given that we could generate exactly reproducible tactile inputs, we found a differential range of inter-trial variability between neurons. Hence, the intracellular recordings of the time-varying synaptic inputs works as a looking glass into the state variations of the network the neuron is connected to. We found that each neocortical neuron to some extent is unique with respect to how internal state variations affect their responses to given tactile input patterns.

Disclosures: **J. Norrlid:** None. **J.M.D. Enander:** None. **H. Jörntell:** None. **H. Mogensen:** None.

Poster

221. Touch: Thalamic-Cortical Processing

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Program #/Poster #: 221.14/J16

Topic: D.04. Somatosensation – Touch

Support: Boğaziçi University BAP Project: 17XP2
TÜBİTAK grant no: 117F481 under European Union's FLAG-ERA JTC 2107 project GRAFIN

Title: Predicting psychophysical response from multi-electrode spike recordings in rat SI cortex

Authors: *S. ÖZTÜRK, B. GÜÇLÜ;
Inst. of Biomed. Engin., Boğaziçi Univ., İstanbul, Turkey

Abstract: The performance of cortical neuroprostheses for providing sensorimotor function depends on decoding spike data from related brain areas and encoding feedback signals into artificial stimulation. In this preliminary study, multiunit spike activity was recorded from the hindpaw representation of SI cortex in 2 awake behaving rats by using 16-channel tungsten microwire array electrodes. This cortical area contains both sensory and motor information. The rats performed a psychophysical yes/no detection task based on 40-Hz vibrotactile stimuli (duration: 0.5 s, amplitude: 200 μm) applied to their hindpaw glabrous skin. Raw spike recordings were sorted offline by using the K-means algorithm (cluster $L_{ratio} < 0.5$). Average firing rates were calculated for the stimulus duration (R_d) and for the 0.5-s interval before the stimulus (R_b) in the stimulus-on trials. The same time windows were also used in the stimulus-

off trials to calculate R_d and R_b. There were significant changes from R_b to R_d in daily sessions. In general, R_d was mostly lower than R_b which implied a suppression of activity with the expectation of the stimulus. When the neural activity measures (R_d-R_b and R_d) were grouped based on psychophysical responses (hit, miss, false alarm, correct rejection), significant differences were found. For both rats, R_d's for hit and false alarm trials were respectively higher than those for miss and correct rejection trials. This shows that higher neural activity during the stimulus (or expected stimulus) interval, i.e. higher R_d might have induced more hits (or false alarms). Regarding individual task sessions, significant correlations were found between R_d and false alarm rate (Rat #1: r=0.49, p=0.047), and between R_d-R_b and hit rate (Rat #2: r=0.58, p=0.038). Next, by using the prior information of stimulus on/off conditions and the average firing rates (R_b and R_d) from each multiunit cluster, a prediction model was established based on logistic regression. The model could predict the psychophysical responses with medium-high accuracies (stimulus-on trials, Rat #1: 73%, Rat #2: 75%; stimulus-off trials, Rat #1: 65%, Rat #2: 80%). In the future, more features will be incorporated into the model for trial-by-trial prediction, which can be implemented for neuroprosthetic applications.

Disclosures: S. Öztürk: None. **B. Güçlü:** None.

Poster

221. Touch: Thalamic-Cortical Processing

Location: Hall A

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Topic: D.04. Somatosensation – Touch

Support: Boğaziçi University BAP Project: 17XP2
TÜBİTAK grant no: 117F481 under European Union's FLAG-ERA JTC 2107 project GRAFIN

Title: Evoked local field potentials from the hindpaw representation of rat SI cortex due to vibrotactile stimulation of the glabrous skin

Authors: *B. GÜÇLÜ, F. T. DUVAN;
Inst. of Biomed. Engin., Boğaziçi Univ., İstanbul, Turkey

Abstract: We previously showed that single neurons in the hindpaw representation of rat SI cortex mostly generate spikes at the onset of vibrotactile stimulation on the glabrous skin. This significantly reduces the intensity information encoded by single neurons. In the current study, we recorded evoked local field potentials by using 16-channel flexible surface electrodes with platinum sites (site diameter: 200 μm). Neural activity was obtained epidurally from the cortical surface of the SI hindpaw representation in 14 anesthetized Wistar albino rats. The vibrotactile stimuli consisted of bursts of sinusoidal (5, 40, and 250 Hz) mechanical displacements (duration:

0.5 s, amplitude range: ~20-270 μm) applied on the contralateral/ipsilateral hindpaw glabrous skin. Similar to single neuron spike data, evoked potentials (0.1-1 mV) were generated with the onset of the contralateral stimulus, and they lasted usually for about 0.1-0.2 s. At high 5-Hz stimulus intensities, evoked potentials were periodic and extended to the stimulus duration (i.e. frequency-following response). The spread of evoked potentials as recorded in different electrode channels was consistent with the cortical projection of tactile information. Ipsilateral stimulation mostly did not produce evoked potentials, but sometimes changed the background activity due to interhemispheric connections. In order to study intensity coding, the root-mean-square values of surface potentials were calculated in two time windows: 1-s interval before the stimulus onset (V_b) and 0.1-s interval starting with the stimulus onset (V_o). Evoked response measure (V_o-V_b), averaged across electrode channels, increased as a function of contralateral stimulus amplitude, but also depended on frequency. A statistical linear mixed model with random effects was established based on fixed factors (stimulation side, stimulus frequency), a covariate (stimulus amplitude), and random intercepts due to subjects. There were significant main effects of contralateral vs. ipsilateral stimulaton ($p<0.001$), of the frequency conditions ($p=0.011$), and of the stimulus amplitude ($p=0.001$) on the average response measures. Post-hoc tests showed that 250-Hz stimulus produced significantly higher channel-average V_o-V_b values than those from 5- and 40-Hz stimuli. However, the channel-average V_o-V_b values from 5- and 40-Hz stimuli were not statistically different. The results suggest that intensity information is indeed represented in a population of activated neurons. Cortical surface potentials based on population activity can be used for decoding sensorimotor information in neuroprosthetic applications.

Disclosures: **B. Güçlü:** None. **F.T. Duvan:** None.

Poster

221. Touch: Thalamic-Cortical Processing

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Topic: D.04. Somatosensation – Touch

Support: RO1 NS085121
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P30NS050274

Title: Divergent inputs to different classes of layer 6 corticothalamic neurons in primary somatosensory cortex

Authors: *C. M. WHILDEN, M. CHEVEE, S. P. BROWN;
Neurosci., Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: In a standard model of the neocortex, primary sensory cortical areas receive sensory information from the periphery via the thalamus. The deepest layer of the cortex, layer 6 (L6), in turn generates a large feedback projection to the thalamus. Studies in several model organisms indicate that layer 6 corticothalamic neurons (L6CThNs) are composed of more than one class of neuron. Here, we test whether different populations of L6CThNs receive synaptic inputs from distinct sets of presynaptic neurons. We hypothesize that these L6CThN cell types are embedded in different neural circuits, and that understanding these differences will generate new insights into the function of corticothalamic circuitry.Morphological and transcriptional findings indicate the existence of two populations of L6CThNs in primary sensory areas in rodents. Using the barrel cortex as a model, we focused on these two types of L6CThNs, both of which express Cre recombinase in Ntsr1-Cre mice. A population in upper L6 sends axons to the ventral posterior medial nucleus of the thalamus (VPM) while a population in lower L6 sends axons to both VPM and the posterior medial nucleus of the thalamus (POm; Bourassa et al, 1995; Chevée et al, 2018).To first compare the axonal projections of these two cell types, we used a viral strategy to bias expression of YFP in VPM-only or VPM/POm L6CThNs. We found that small groups of VPM-only L6CThNs project vertically to narrow regions in layer 4 and to VPM. The axons of small groups of VPM/POm L6CThNs, in contrast, ramified widely in layer 5A and more broadly in VPM and POm.To compare the presynaptic inputs to these two populations of L6CThNs, we used a viral strategy based on the rabies virus. We observed a consistent input pattern to VPM/POm L6CThNs from VPM, POm, and the ventrolateral nucleus of the thalamus, layer 5A of contralateral cortex, and local neurons in infragranular cortex. Inputs to VPM-only L6CThNs appeared to differ in several ways. They received input from different thalamic sources than VPM/POm L6CThNs and did not receive input from contralateral layer 5A. These data indicate these two populations of L6CThNs are embedded in different neural circuits and further suggest that their roles in sensory perception are distinct

Disclosures: C.M. Whilden: None. **M. Chevee:** None. **S.P. Brown:** None.

Poster

221. Touch: Thalamic-Cortical Processing

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Topic: D.04. Somatosensation – Touch

Support: NHMRC, APP1085708
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Viertel Fellowship

Title: Neural correlates of anticipation in mouse primary somatosensory and prelimbic cortices

Authors: *R. GUZULAITIS[1], L. M. PALMER[2];
[1]The Florey Inst. of Neurosci. and Mental Hlth., [2]Florey Inst. of Neurosci. and Mental Hlth., Florey Institute, Univ. of Melbourne, Melbourne, Australia

Abstract: Anticipation is a feeling of expectation about something that is going to happen in the near future. Since it binds past sensory experiences with future sensation, here we tested whether sensory processing in the cortex is influenced by anticipatory signals. Patch clamp whole-cell recordings were performed in the primary somatosensory and prefrontal (prelimbic) cortices in awake behaving mice during a cue-based anticipation task. Mice were trained to respond to a tactile sensory stimulus following the presentation of an auditory cue. During this task, the activity of pyramidal neurons in the primary somatosensory cortex was tightly linked to behavioural performance, with large voltage response during correct behaviour. During the auditory cue presentation, ramping voltage activity preceding the tactile stimulus occurred in trials with cue-initiated animal movement. Conversely, pyramidal neurons in the prefrontal cortex were strongly activated not only during performance of the animal but also during the anticipatory phase of the task (cue presentation). These findings show that both the somatosensory and prefrontal cortices encode performance of a sensory-based task whereas the prefrontal cortex also has strong anticipatory voltage signals.

Disclosures: R. Guzulaitis: None. **L.M. Palmer:** None.

Poster

221. Touch: Thalamic-Cortical Processing

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Topic: D.04. Somatosensation – Touch

Support: NIH Grant NS07798
NIH Grant NS085171

Title: Calcium-activated TRPM4 conductances mediate persistent firing in neurons of the thalamic reticular nucleus

Authors: J. O'MALLEY[1], G. S. STEPHENS[2], F. SEIBT[1], J. CHIN[2], *M. BEIERLEIN[1];
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Abstract: Slow-wave sleep (SWS) is characterized by slow (0.1 - 4 Hz) oscillations in both neocortex and thalamus, with neurons fluctuating between bouts of intense action potential activity (UP states) and almost complete silence (DOWN states). In neocortex, slow oscillations and UP states are generated and sustained by activity in highly recurrent local networks. By

contrast, neurons in the thalamus and the thalamic reticular nucleus (TRN) are thought to generate slow oscillations and UP states with persistent firing (PF) in a cell-intrinsic manner. PF is thought to require postsynaptic mGluR activation and to be sustained by a plateau potential mediated by the window component of the T-type calcium current and a calcium-activated nonselective cation current (I_{CAN}). However, the molecular identity of I_{CAN} and the cellular and network contributions to slow thalamic oscillations remain unknown. Using whole-cell and cell-attached recordings from thalamic brain slices derived from adult mice, we found that brief depolarizing current steps led to PF in about one third of TRN neurons, while neurons in the neighboring ventrobasal nucleus of the thalamus (VB) never displayed PF. PF in the TRN required activation of T-type calcium channels and was at least in part mediated by a TTX-insensitive Na current. We found that antagonists for calcium-activated TRPM4 conductances reliably blocked PF, whereas genetic deletion of a number of TRPC conductances had no effect on PF. In slices preserving intrathalamic connectivity, neurons in the TRN often displayed robust oscillatory bouts of PF at 0.5 - 2 Hz. Oscillations were insensitive to block of postsynaptic mGluRs but required AMPAR-mediated synaptic transmission. In preliminary studies, we employed a mouse model of Alzheimer's disease (AD) to determine alterations in TRN neuronal firing. We found that in transgenic mice that overexpress mutant human amyloid precursor protein (APP) and produce high levels of Aβ, the percentage of TRN cells showing PF was reduced, accompanied by a reduction of TRPM4 mRNA. Our results suggest that PF in the TRN is triggered by calcium influx through T-type calcium channels, in turn leading to the activation of calcium-dependent TRPM4 conductances. Moreover, while PF was generated in a cell-intrinsic manner, synaptic networks interconnecting TRN and VB were found to be essential for slow oscillatory thalamic activity.

Disclosures: **J. O'Malley:** None. **G.S. Stephens:** None. **F. Seibt:** None. **J. Chin:** None. **M. Beierlein:** None.

Poster

221. Touch: Thalamic-Cortical Processing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 221.19/J21

Topic: D.04. Somatosensation – Touch

Support: NIH Grant U01MH10602
NIH Grant U01NS094302
NIH Grant R01NS104928

Title: Thalamic modulation and the shaping of cortical sensory representations in the awake and anesthetized mouse

Authors: *P. Y. B. BORDEN[1], N. C. WRIGHT[1], A. J. SEDERBERG[2], C. WAIBLINGER[1], B. HAIDER[4], G. B. STANLEY[3];
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Abstract: A large body of work, primarily in the anesthetized animal, has demonstrated that thalamic firing properties (or "state") can dynamically shape ongoing and sensory-evoked cortical activity. In particular, ongoing and sensory-evoked firing rate, single-unit bursting, and population synchrony in the thalamus have been shown to shape the downstream cortical sensory response. Yet this conceptual model of thalamic gating has been largely unexplored in the awake animal, and likely plays a major role in modulating sensory processing. Here, we measured and optogenetically manipulated the thalamus, while tracking cortical activity in the vibrissa pathway of the awake mouse. We recorded single- and multi-unit activity in the ventral posteromedial nucleus (VPm) of thalamus, simultaneous with either mesoscale voltage imaging (using the GEVI ArcLight) or laminar probe recordings in S1. We found that optogenetic hyperpolarization of VPm amplifies ongoing and whisker-evoked thalamic firing by engaging putative t-type calcium channel bursting. Counterintuitively, despite the enhanced thalamic sensory response, the downstream S1 response decreased in amplitude and spatial extent with thalamic hyperpolarization. In addition, the temporal dynamics of the cortical response changed as a function of light delivery levels. We hypothesized that this unexpected trend in the S1 response reflected the effects of elevated ongoing VPm firing prior to sensory stimulation. We tested this hypothesis by repeating these experiments in the lightly-anesthetized mouse, in which baseline firing rates in VPM are dramatically reduced. In these experiments, both thalamic and S1 sensory responses increased with thalamic hyperpolarization, which had been masked by more complex dynamics in the awake state. This suggests that there is indeed a complex interplay between thalamic state and cortex, involving not only sensory-evoked thalamic firing, but also thalamocortical synaptic depression (due to thalamic firing prior to sensory stimulation) and intracortical inhibitory dynamics (reflected in the time course of the cortical response). Taken together, this work emphasizes a complex interaction between thalamus and cortex that dynamically gates sensory signaling that we are only just beginning to understand, and this likely plays a key role in shaping sensory percepts.

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Poster

221. Touch: Thalamic-Cortical Processing

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ANR-16-CE37-0003
ANR-17-CE37-0009
Labex Memolife

Title: Cerebellocortical coupling via the medial posterior thalamus: A role in whisker-dependent texture discrimination

Authors: *H. BABA AISSA[1], A. VARANI[1], J. L. FRONTERA[1], M. DIANA[2], J. CARCAUD[1], T. TARPIN[1], P. COULON[3], C. LENA[1], D. POPA[1];
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Abstract: Increasing evidence indicates that the cerebellum is involved in complex brain processes, such as sensorimotor integration in active sensory discrimination. Here we focus on the involvement of cerebellum in somatosensory pathways.
We first show using rabies virus injected in the primary somatosensory cortex that this area receives inputs from the cerebellar nuclei, the latency of infection of cerebellar nuclei neurons being consistent with a disynaptic cerebello-cortical pathway. Second, we verified that primary sensory cortex neurons are activated by cerebellar nuclei stimulation, with a short latency similar to the responses observed in primary motor cortex; this is also consistent with the existence of a disynaptic pathway between the cerebellum and primary somatosensory cortex. Third, we sought potential relays between the cerebellum and sensory and motor cortical areas by combining injections of anterograde markers in the cerebellar nuclei and retrograde tracers in the cortical areas. We found abundant cerebellar terminals in areas of the thalamus projecting to these cortices. Notably, we observed that the area of the medial Posterior thalamus containing neurons projecting to sensori-motor cortices received abundant cerebellar afferents.
We then examined in slices the functionality of the cerebellar afferents to the Pom and found using optogenetic stimulation that they generate large synaptic currents in Pom neurons, indicating that the cerebellum provides strong inputs to the Pom thalamus. In order to test whether the cerebellar afferents to the Pom contribute to the active sensory discrimination, we inhibited the cerebellum-Pom neurons using a targeted chemogenetic approach in a novel-object recognition task, and we observed a disruption of texture discrimination, but not of object discrimination, Altogether, these anatomical, functional and behavioral results suggest the existence of a disynaptic pathway from the cerebellum to the sensory cortex, and that this pathway is important for allowing texture discrimination, thus highlighting the role of the cerebellum in sensory information processing.

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Poster

221. Touch: Thalamic-Cortical Processing

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Topic: D.04. Somatosensation – Touch

Support: R01 NS069679
R01 NS094659
F31 NS098670
F32 NS092357
T32 EY013933

Title: Activity in secondary sensory thalamus during whisker-based detection of relevant objects

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Abstract: Out of the array of objects in our environment, the brain selectively identifies and responds to those that are most important. We seek to understand this process in mice, who actively move their whiskers onto objects to localize and identify them. In whisker-mediated touch and other senses, primary sensory cortex, the first point of processing within the cerebral cortex, receives input from both primary and secondary thalamic nuclei of the same sensory modality. Whereas primary thalamic nuclei are thought to convey spatiotemporally precise information to the cortex, the responses of secondary nuclei are poorly tuned and less well understood. We hypothesized that secondary thalamic nuclei process stimuli that are important in the current behavioral context. To test this, we developed a novel whisker-based detection task in which we present a “target” pole (indicative of reward) in one location and a physically identical “distractor” pole (not predictive of reward) in a different location. Mice successfully learned to associate the presence of the target pole with reward. They whisked more when the target was present but did not whisk more when only the distractor was present. We monitored neural activity using two-photon microscopy of the calcium indicator GCaMP6f through gradient index (GRIN) lenses implanted into secondary thalamic nuclei. To determine the specificity of these responses, we compared activity in the somatosensory secondary thalamus (the posterior medial nucleus, POm) and the visual secondary thalamus (the lateral posterior nucleus, LP). Surprisingly, we observed that the visual nucleus is differentially modulated by trial conditions during this whisker-mediated task.

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Poster

221. Touch: Thalamic-Cortical Processing

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Topic: D.04. Somatosensation – Touch

Support: NIH Grant NS061963
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Title: Cortico-thalamo-cortical loops of forelimb somatosensory cortex

Authors: *K. GUO[1], N. YAMAWAKI[1], M. TAPIES[1], G. M. SHEPHERD[2];
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Abstract: The cellular specificity of excitatory synaptic connections from cortex to thalamus is an important but incompletely understood aspect of somatosensory pathways. We used a combination of tools to dissect these connections, focusing on forelimb primary somatosensory cortex (S1) in the mouse. The results delineate multiple recurrent loops. First, inputs from corticothalamic (CT) neurons in S1 layer 6 strongly excite S1-projecting neurons in the ventral posterior lateral (VPL) nucleus. Second, the same S1-CT axons also excite S1-projecting neurons in an S1-associated subregion of the posterior nucleus (PO). Third, thalamic branches of S1 pyramidal-tract (PT) neurons excite the same group of S1-projecting PO neurons. In contrast, M1-projecting PO neurons, located in a different subregion of PO, generally receive little or no input from either S1-CT or S1-PT axons. Together with previous evidence for robust M1-CT and M1-PT connections to these M1-projecting PO neurons, our results show that the cortico-thalamo-cortical circuits of CT and PT neurons in these somatosensory pathways are configured to support recurrent signaling via area-specific subregions of PO.

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Poster

221. Touch: Thalamic-Cortical Processing

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Topic: D.04. Somatosensation – Touch

Support: NIA Grant F31AG057155

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Human Frontiers Grant RGP0036

Title: Astrocytes modulate sensory-evoked neuronal network activity

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Abstract: In contrast with the widely accepted paradigm that brain function is principally mediated by neurons, astrocytes are emerging as important cells in brain physiology because they interact with neurons at tripartite synapses. Indeed, besides their classical homeostatic roles, astrocytes respond to neurotransmitters with rises in internal calcium levels that in turn release gliotransmitters that regulate synaptic and neuronal functions. While astrocyte calcium and consequent synaptic regulation has been largely documented at the cellular level, astrocyte network activity and its impact on neuronal network function has been minimally explored. We have investigated this issue by combining simultaneously acquired two-photon microscopy *in vivo* to monitor astrocyte activity and electrocorticogram (ECoG) recordings to monitor neuronal network activity in the somatosensory cortex (S1) during hindpaw stimulation. We observed astrocyte sensory responses that begun in astrocytic microdomains and expanded to the soma after reaching a spatial threshold. By delivering trains of stimuli at different intensities, durations and frequencies, we found that astrocyte populations respond in a reliable and stimulus dependent manner. Through the combined use of calcium imaging and ECoG we identified that sensory evoked astrocyte calcium responses were correlated with rises in cortical gamma power (30-80 Hz), but not low frequency delta activity (1-4 Hz). This sensory-evoked cortical gamma activity was increased in transgenic mice that had impaired astrocyte calcium signaling. In contrast, specific activation of astrocytes selectively expressing Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) reduced the sensory-evoked gamma power. We conclude that cortical astrocytes respond to sensory inputs with calcium rises that in turn regulate sensory-evoked neuronal network activity.

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Poster

221. Touch: Thalamic-Cortical Processing

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Topic: D.04. Somatosensation – Touch

Support: NIH R01MH112267

Title: Pupil-linked arousal dependent sensory processing in awake somatosensory thalamus

Authors: *Z. FAZLALI, Y. ZHANG, C. RODENKIRCH, Q. WANG;
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Abstract: The thalamus receives cortical, subcortical, and neuromodulatory inputs. Recent experimental evidences indicate that the thalamus is a critical stage for sensory processing as it dynamically gates information flow to the cortex. Sensory processing is heavily dependent upon behavioral states, including arousal and attention. For example, previous work has demonstrated that changes in pupil size covary with the membrane potential fluctuations of cortical neurons and are able to account for cortical response variabilities, suggesting that arousal indexed by pupil size (i.e. pupil-linked arousal) exerts influences on cortical information processing. However, how pupil-linked arousal modulates thalamic sensory information remains poorly understood. Here we performed single-unit recording from the ventral posteromedial nucleus (VPm) and thalamic reticular nucleus (TRN) while simultaneously measuring pupil size in awake, head-fixed rats. The firing rate of neurons in both VPm and TRN was positively correlated with pupil size. Interestingly, thalamic burst firing, a firing pattern thought to be associated with anesthesia or sleep, was present in awake thalamus. In addition, the rate of the burst firing was found to be dependent on pupil size, with significantly higher bursting rates occurring during time periods when the pupil was relatively constricted. Bursting-event-trigged pupil size revealed a decrease in pupil size around bursts with pupil size reaching a minimum approximately 1 s after the burst events. Further, during constricted-pupil time periods LFP recordings from both the VPm and TRN showed an increase in the power of thalamocortical spindle oscillation (7-14 Hz) while the power of gamma oscillation (30-80 Hz) was decreased. Surprisingly, the average VPm and TRN spike count in response to punctate whisker stimulation was higher during low-arousal states than in high-arousal states. Repetitive whisker stimulations delivered at different frequencies resulted in rapid adaptation of firing rate in a frequency dependent fashion for both the TRN and VPm. Consistent with previous results in anesthetized animals, sensory adaption was stimulus intensity dependent in awake thalamus, evidenced by a smaller adaptation ratio (e.g. ratio of number of spikes evoked by the last deflection to that evoked by the first deflection) for high intensity stimulus than low intensity stimulus. Moreover, strength of adaptation was linked with pupil-size as low-arousal, constricted-pupil time periods exhibited a larger adaptation ratio. Taken together, these results suggest that thalamic sensory processing is heavily dependent upon pupil-linked arousal.

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Poster

222. Auditory Processing

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Topic: D.06. Auditory & Vestibular Systems

Support: ANR Grant 16-CE17-0016
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Vinci, Université Franco Italienne

Title: An active multisensory motor strategy to sound localization in virtual reality

Authors: *C. VALZOLGHER[1,2], G. VERDELET[1], R. SALEMME[1,4], L. LOMBARDI[3], A. FARNÉ[1,4,2], F. PAVANI[2,1,3];
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Abstract: Several lines of research have shown that re-learning to localize sound in space is possible throughout life. Multisensory and motor cues can play a key role in re-learning associations between auditory cues and space. In this study, we tested if active movements towards sounds can promote acoustic space re-learning across four successive monaural listening blocks. Participants performed a sound localization task, in binaural and monaural listening conditions, while immersed in a virtual reality scenario showing 17 speakers at ear level. Specifically, we compared this active interaction (reaching) with a control condition in which participants were exposed to identical audio-visual stimulations, but they read labels to identify sound sources (naming). Twenty-eight participants (14 for each group) localized a white noise amplitude modulated (4 Hz) continuous sound (60 dB SPL) in 5 successive blocks: one binaural block, followed by 4 monaural listening blocks (left ear plugged). The sound continued until the participant correctly identified its position; if the first response was wrong the correct speaker flashed guiding participants to the correct sound location. Critically, participants were free to move the head during the task. Thus, we measured both performance indices and head movements. Most interesting observations are: (1) our novel multisensory training strategy determined performance improvement, which emerge rapidly across successive blocks within a single testing session; (2) compared to naming, reaching induced faster and larger improvements across monaural trials; (3) error reduction was accompanied by wider head-movements, whose extension changed as a function of target eccentricity; (4) performances improvement and head-movements extension correlate, especially for reaching, suggesting a role of active listening in the observed advantage for this task.

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Poster

222. Auditory Processing

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Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant DC003180

Title: Imaging the representation of sound location in the auditory cortex of awake marmoset

Authors: *C. CHEN, X. SONG, Y. GUO, X. WANG;
Dept Biomed Engin, Johns Hopkins Univ. Sch. Med., Baltimore, MD

Abstract: Despite several decades of research, the nature of neural representation of sound location in auditory cortex remains unclear. Previous studies have failed to identify any maps or organization of spatial representation in mammalian auditory cortex. A prevailing hypothesis of cortical spatial processing is the distributed population coding, supported by the evidence that neurons responding broadly to sound locations on the contralateral hemifield. Recent electrophysiology studies in awake marmosets showed spatially highly selective cortical neurons and diverse spatial receptive fields across cortical surface. However, single or multi-unit electrophysiology method has limited power to evaluate both local and global organizations of cortical representation of sound locations. In the present study, we took the advantage of the flat brain of the marmoset, a highly vocal New World monkey, and used wide-field optical imaging methods to investigate the neural representations of sound location in auditory cortex in awake condition. The sound stimuli were Gaussian noises delivered in free-field on the horizontal plane from both contralateral and ipsilateral locations. Using wide-field calcium imaging method, we observed systematic changes in cortical response patterns as the spatial location varied along the azimuth axis. The pixels corresponding to the maximum responses to one of the tested sound locations formed clear clusters in the core region of the auditory cortex. These clusters were relatively stable across multiple sound levels. In addition, the pixels that showed strong calcium response to particular spatial stimuli also showed large hemodynamic changes as revealed by intrinsic imaging. These observations suggest that cortical neurons with similar spatial receptive fields may form local clusters and the possibility of an orderly organization of spatial representation in marmoset auditory cortex.

Disclosures: C. Chen: None. **X. Song:** None. **Y. Guo:** None. **X. Wang:** None.

Poster

222. Auditory Processing

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Topic: D.06. Auditory & Vestibular Systems

Support: Hong Kong Health and Medical Research Fund (HMRF) Project No. 06172296
Shenzhen Science Technology and Innovation Committee Grant No. JCYJ20180307124024360

Title: Temporal weighting functions for binaural cues in rats

Authors: *K. LI[1], C. H. K. CHAN[1], A. P. MISHRA[1], J. W. H. SCHNUPP[1,2];
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Abstract: The "temporal weighting function" (TWF) quantifies how strongly lateralisation judgments in spatial hearing are influenced by the onset, middle, or offset of a sound respectively. They are usually measured in psychoacoustic experiments using binaural click trains, in which individual clicks differ in their binaural cue values. Human listeners tend to show a strong "onset bias" in such studies (Brown and Stecker, 2010; Stecker and Hafter, 2002; Stecker, 2014). While the shape of the TWF is likely to be similar in other mammals, to the best of our knowledge, this has not previously been shown. To measure the TWF for rats, we performed psychoacoustic experiment using click train stimuli with jittered interaural time differences (ITDs). Four 8-week old female Wistar rats performed a two-alternative forced choice near-field lateralization task. The experiments involved randomly interleaved "honesty trials" (80% of trials), and "probe trials" (20%). We then computed TWFs by performing a multiple regression of the ITD value against the animals' "left" or "right" responses for the probe trials only. ITD TWFs were measured in this way for click rates of 20, 50, 300 and 900 Hz. Onset dominance was observed across all click rates for all rats. The weights on the later clicks tended to increase as the click rate decreased. In a few cases, significant weight on the last click (offset) was also found, mainly in the lower frequencies. Our findings demonstrated that the auditory process in rats is similar to human, showing clear onset dominance and recency effect. The rat is therefore shown to be an easily accessible and feasible animal model for related auditory research.

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Poster

222. Auditory Processing

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Topic: D.06. Auditory & Vestibular Systems

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Title: Evaluating nonlinear auditory steady-state responses in EEG using multi-spectral phase-coherence analysis

Authors: L. WANG[1], E. NOORDANUS[2], Y. YANG[3], *J. A. VAN OPSTAL[4];
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Abstract: Background: A periodic complex auditory stimulus can evoke an auditorysteady-state response (ASSR), which can be measured on cortical EEG signals. The typical ASSRs (i.e., EEG components withthe same frequency as the modulations in the envelope) are used as an objective assessment of auditory function and hearing loss. The ASSR can be viewed as a nonlinear response of the auditory system. However, the underlying nonlinearity in the auditory pathway needs to be further revealed by using more complex stimuli and by using nonlinear systems identification methods.**Goals:** We aimed (1) to evaluate the nonlinear ASSRs, i.e., the steady-state spectral components in the EEG that do not exist in the stimuli; (2) to reveal the underlying nonlinearity in the auditory pathway.**Experiments:** We performed two experiments with different four-tone stimulus complexes(i.e., multiple sinusoidal stimuli on both ears, simultaneously presented) on ten normal-hearing adults. Each subject was measured twice on different days. In each measurement, the four-tone stimuliwith different intensities werefed to both ears, while measuring the scalp EEG from 64 electrodes.**Analytic methods:** We used both the traditional spectrum-analysis methods and the multi-spectral phase-coherence (MSPC) method to identify the significance of evoked ASSRs, and their nonlinear interactions. MSPC quantified the dominant high-ordernonlinearities in a statistical manner and estimated the potential latencies between the stimuli and certain components in the ASSR. Source estimation was further performed by using sLORETA.**Results:** The results from MSPC and the traditional methods revealed highly consistent frequencies with significant ASSRs. These frequencies are subsets of 2^{nd}-order, 4^{th}-order and 6^{th}-order nonlinear system responses to the stimuli. The potential latencies estimated by MSPC based on two dominant ASSR components (around 40 Hz and evoked from different ears)were around 118 (±4) ms, while the latency of ASSRs with

higher frequencies (around 80 Hz) werearound27 (±2) ms across ten subjects. The EEG electrodes above the central-frontal brain and the right temporal lobe areas showed the strongest ASSRs. The source estimation results suggested that maximum activation brain regions were affected by the dominance of monaural or binaural interactions.**Conclusions:** ASSRs evoked by multi-sine stimuli can be used to assess underlying nonlinearities in the human auditory system. The quantified nonlinearity can potentially serve as a new objective assessment of auditory function.**Acknowledgments:**TTW-NWO grant Perspectief (LW,EN), Horizon 2020 ERC Adv grant ORIENT (AJVO)

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Poster

222. Auditory Processing

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Topic: D.06. Auditory & Vestibular Systems

Support: BBSRC Grant BB/R004420/1

Title: Allocentric sound localization in ferrets

Authors: *S. M. TOWN, J. K. BIZLEY;
UCL Ear Inst., London, United Kingdom

Abstract: The ability to localize sounds is critical for both human and non-human hearing. Humans can describe sound position within coordinate frames defined by the observer (head, eyes, etc.) or environment. However, it's unclear if other animals perceive sound location in multiple reference frames and particularly whether animals can report world-centered sound location. Neurons within the ferret auditory system represent both head-centered and world-centered (allocentric) sound location, suggesting that non-human world-centered auditory perception could exist.
Here, we tested if ferrets could report world-centered sound location in a two-choice allocentric sound localization task where subjects reported the positions of one of two speakers located at opposite sides of a test arena. Trials were initiated and test sounds (250 ms broadband noise) presented while the animal was at a central platform located equidistant between sound sources. Following sound presentation, animals responded at one of two response ports that were not co-located with sound sources. Across trials, the platform was rotated at 30° intervals across 360° to prevent use of sound location cues relative to the head or eyes.
We found that ferrets (n=2) could successfully report sound source location at each platform rotation tested and thus discriminate sound location in the world independently of sound angle relative to the head. Furthermore, generalization of sound location across platform angles

occurred immediately after rotation, indicating that subjects did not rapidly re-learn head-centered cues to solve the task. Rather, our results show that ferrets developed a rule-based strategy utilizing the absolute position of sounds in the world, independent of head orientation. Finally, presentation of probe sounds at novel, untrained locations revealed that ferrets judged sound locations on a continuum across space rather than as discrete spatial categories.
Together, our work suggests that like humans, other animals can also perceive sound location in allocentric coordinates. This opens the door for future recording of neurons in the auditory system to understand how neural circuits support coordinate frame transformation of sound space across the brain.

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Poster

222. Auditory Processing

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Program #/Poster #: 222.06/J32

Topic: D.06. Auditory & Vestibular Systems

Support: NIH NIDCD R01 DC011555

Title: Binaural hearing in the naked mole-rat

Authors: *E. MCCULLAGH[1], J. PEACOCK[1], A. KLUG[1], T. PARK[2], D. J. TOLLIN[1]; [1]Dept. of Physiol. and Biophysics, Univ. of Colorado Anschutz, Aurora, CO; [2]Biol., Univ. of Illinois Chicago, Chicago, IL

Abstract: The Naked mole-rat (*Heterocephalus glaber*) is increasingly being used as a laboratory mammal due to its resistance to aging and cancer, as well as other peculiar traits. The mole-rats live in eusocial, underground colonies of around 80 animals. The underground tunnels in which they live limit the range of acoustical information the animals are exposed to. Sounds propagate well through the burrow, but there are very limited sound localization cues. However, the mole-rats do produce a wide range of vocalizations for communication, suggesting that sound, and thus hearing, plays an important role in their lives.
Previous studies have suggested that mole-rats have a degenerate hearing system. Published behavioral measurements show poor hearing thresholds, limited high frequency hearing, and poor sound localization abilities. And published anatomical measurements have shown intraspecific variations in middle and inner ear structures. Further studies have also shown that the mole-rats lack the HCN1 ion channel from binaural nuclei, which contributes to the integration of binaural inputs in the brainstem, thus potentially impeding their ability to effectively localize sounds.
In order to provide a more comprehensive understanding of hearing and sound localization

ability in mole-rats, we made various anatomical and physiological measurements. Specifically, we made measurements of the binaural interaction component (BIC) of the auditory brainstem response (ABR), which is a non-invasive electroencephalographic signature of neural processing of binaural sounds by brainstem neurons. We report that naked mole rats do have a measurable BIC of the ABR similar to other laboratory species commonly used for sound localization research. However, it is markedly variable across individuals. On the other hand, the BIC varied with interaural time difference, suggesting an ability to localize sound. Additionally, we performed histological analysis to investigate the underlying neuroanatomy of the brainstem of these peculiar mammals. In conclusion, the naked mole rat may have a more refined auditory brainstem than previously shown, making them an interesting species for future sound localization research.

Disclosures: E. McCullagh: None. **J. Peacock:** None. **A. Klug:** None. **T. Park:** None. **D.J. Tollin:** None.

Poster

222. Auditory Processing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 222.07/J33

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant DC007690
NIH Grant NS104911

Title: The effect of anticipated cue reliability on neural adaptation and novelty detection in barn owls

Authors: *K. SHADRON, R. FERGER, J. L. PENA;
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Abstract: The brain actively updates the representation of the environment. An open question about this function is whether adaptation is weighted by the predicted statistics of sensory information. Here we asked whether anticipated cue reliability affects the rate of adaptation in the auditory system of the barn owl.
The midbrain of the barn owl contains a map of auditory space, which uses the interaural phase difference to compute sound location in azimuth. Previous work showed that space-specific neurons in this map are tuned to the frequency range that is most reliable for its preferred location. This effect is due to the acoustical properties of the head, causing higher frequencies to convey interaural phase difference (IPD) more reliably in frontal space and lower frequencies in the periphery in the presence of concurrent sound. We hypothesized that adaptation would be optimized for anticipated reliability, thereby causing a bias in cases of expected low reliability.

We also sought to determine if this would lead to a similar bias in novelty detection, where adaptation has been implicated as a potential mechanism. We measured the pupillary dilation response, an orienting response that adapts upon repetition of a stimulus and readily recovers when novel stimuli are presented. Tones of different frequencies and IPD were repeatedly presented to awake barn owls through earphones. This approach was used to assess whether PDR adaptation was correlated with anticipated IPD reliability despite actual IPD reliability being unchanged in sounds delivered through earphones. To assess the strength of the novelty detection, a deviant in auditory location was then presented to elicit a recovery of the PDR. We found that novelty detection was more robust when the anticipated reliability was higher. To assess this question at the neural-population level, we conducted recordings of multiple midbrain neurons using a microelectrode array. Adapter and test stimuli were used to assess population and activity and tuning of individual cells before and after adaptation. Frontal and peripheral neurons were compared to test the hypothesis that anticipated reliable and unreliable stimuli lead to different adaptation rates. Our preliminary results suggest an effect of anticipated statistics on sensory adaptation.

Disclosures: **K. Shadron:** None. **R. Ferger:** None. **J.L. Pena:** None.

Poster

222. Auditory Processing

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Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 222.08/J34

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant NS104911

Title: Competing stimuli in the owl's auditory space map - Evidence supporting a population vector readout

Authors: *R. FERGER[1], M. BECKERT[1], K. SHADRON[1], B. J. FISCHER[2], J. L. PENA[1]; [1]Neurosci., Albert Einstein Col. of Med., Bronx, NY; [2]Seattle Univ., Seattle, WA

Abstract: The map of auditory space in the owl’s midbrain supports accurate sound localization. Ensemble activity and network architecture in this map has been postulated to represent natural statistics, conveyed to behavioral commands through a population vector (PV) readout of the map, which approximates Bayesian statistical inference. We have previously shown that population response profiles match the conditions for a PV readout. Here we present evidence supporting that a PV based model for the readout of the owl's midbrain auditory space map also works when competing auditory stimuli are presented. We performed multi-electrode array (MEA) recordings of responses in the owl's optic tectum to binaurally presented auditory stimuli - conveying sound localization cues such as interaural time difference (ITD). A decoder based on

the PV readout model was used to estimate the stimulus ITD from single trial responses of recorded sub-populations. When two stimuli with different ITDs (i.e. from different directions) were presented, the population vector pointed towards one of the sound sources. This could not be predicted by a simple addition of activity evoked by each stimulus alone. Rather, which stimulus direction is represented by the population depended on the relative saliency of competing stimuli. Both stimulus level and onset timing were used to manipulate saliency. Our decoder was capable of estimating the ITD of the louder sound in a competing stimulus condition (simultaneous onset). This shaping of the population response is consistent with a global inhibition network recently discovered. When two stimuli are presented with slightly different onset times (equal levels), the expectation is that the leading stimulus would also be more salient and, thus, determine the direction represented in the population response. All together, these results show that the PV model can perform accurate localization in complex auditory scenes.

Disclosures: R. Ferger: None. **M. Beckert:** None. **K. Shadron:** None. **B.J. Fischer:** None. **J.L. Pena:** None.

Poster

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Topic: D.06. Auditory & Vestibular Systems

Support: NSF grant BCS-1539376

Title: Neural evidence of location constancy for auditory spectral processing in marmoset auditory cortex

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Abstract: The computational demands for disassociating sound location (‘where') and spectral feature (‘what') information from each other pose a unique problem for audition. In a naturalistic environment, the signal entering the ear canal is the sum of the direct sound wave from the source and attenuated, delayed reflections from nearby surfaces. One well-known example of this is pinnae filtering. The interference between direct and reflected sound waves attenuates and amplifies signal energy in a frequency-dependent manner, depending on the spatial locations of the source. It remains unknown to what extent the location-related spectral filtering affects spectral processing in the auditory system. In this study, we investigated the effects of varying sound source location on the frequency selectivity of individual neurons in the marmoset primary auditory cortex (A1). The experiment first tested the spatial tuning functions of a neuron to best-

frequency tones and broadband noises (100-500 msec duration) over 360 degrees in the horizontal plane. Then the frequency tuning function was evaluated for sound sources presented from four opposite quadrants: contralateral-frontal, contralateral-rear, ipsilateral-frontal, and ipsilateral-rear. We found that cortical neurons across cortical layers show diverse spatial selectivity in the peak direction and tuning width. Varying sound location modulates the response gain of A1 neurons. However, their frequency selectivity, such as harmonicity sensitivity, remains largely unaffected across the four spatial quadrants. These results suggest that A1 frequency tuning remains insensitive to spectral perturbations of pinna filtering. The gain-modulation between spatial vs. spectral tuning appears necessary to the dissociation between ‘what' and ‘where' information to achieve location constancy in auditory object perception.

Disclosures: Y. Zhou: None. **J. Braasch:** None.

Poster

222. Auditory Processing

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Program #/Poster #: 222.10/J36

Topic: D.06. Auditory & Vestibular Systems

Support: ANR-16-CE17-0016

Title: Acoustic space perception: Relating explicit spatial confidence and performance accuracy

Authors: G. RABINI[1], G. LUCIN[2], G. MONITTOLA[1], A. FARNÈ[3,1], D. BALDAUF[1], F. PAVANI[1,2,3];
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Abstract: Humans perceive the surrounding acoustic space primarily by means of sound localisation abilities, which rely on the interpretation of binaural and monaural auditory cues. The analysis of these cues by the auditory system allows the association between sound waves and specific coordinates in the external space. Interestingly, this sound-space correspondence, is altered in conditions of auditory cues perturbation, such as monaural listening. To date, sound localisation performance has been described by indexes of accuracy, biases or variability, with lacking interest in the conscious spatial perception of sounds in space. The principal aim of the present study was to investigate the degree of explicit spatial certainty on sound position during a sound localisation task in binaural and monaural listening condition, with the further aim to relate localisation performance and explicit spatial certainty on sound location. In two behavioural experiments (N=20 each), participants localised single free-field sound sources in

binaural and monaural listening condition, giving explicit ratings of spatial certainty (confidence) on sound position, trial by trial. Results showed performance decrease from binaural to monaural listening, in line with previous literature, with a concomitant decrement of confidence on sound location. Furthermore, different patterns of accuracy-confidence relation emerged both in binaural and monaural listening. In particular, under monaural listening, participants showed both high and low degrees of spatial certainty, regardless the level of accuracy. Intriguingly, perceived sound position with different combinations of performance accuracy and spatial confidence follow specific temporal and spatial distributions. These findings suggest that sound localisation, especially in altered listening condition, is far more complex than showed in previous investigations, and that explicit confidence on sound location could be a fundamental index to characterise the subjective experience of acoustic space perception in humans. Finally, an ongoing MEG study on this paradigm will extend the current findings highlighting the cortical network subtending sound-space correspondence and the interplay with subjective spatial confidence on sound position.

Disclosures: G. Rabini: None. **G. Lucin:** None. **G. Monittola:** None. **A. Farnè:** None. **D. Baldauf:** None. **F. Pavani:** None.

Poster

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Topic: D.06. Auditory & Vestibular Systems

Support: Cocher co.
WISET 2018-092
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NRF 2016R1A2B4016330

Title: Noise effect on the auditory evoked responses to different sound locations in unilateral deafness: Hemispheric asymmetry and relationships to speech perception

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[1]Lab. of Brain & Cognitive Sci. for Convergence Med., [2]Dept. of Otolaryngology, Hallym Univ. Col. of Med., Anyang, Korea, Republic of

Abstract: Objectives: Unilateral deafness complains of sound localization, and it is even more difficult in noise environment. However, the source of the difficulty and the relation of sound localization with noise is still unknown. Therefore, we measured the N1/P2 cortical responses to different sound locations under background noise to compare with quiet condition.
Methods: Eleven unilateral deaf (UD) adults (mean age=53) and nine normal-hearing (NH)

controls (mean age=51) participated. In UD group, five were left-sided and six were right-sided deafness. Speech stimulus to evoke cortical response was CV syllable /ba/ of 450 ms duration. A sound localization task was consisted of speech coming from straight ahead (0^o), and from either 15^o or 60^o to the healthy ear (15^o or 60^o) or deaf ear (-15^o or -60^o). For the noise condition, +5dB of speech- shaped noise was added to the /ba/ stimulus. N1/P2 were analyzed as a function of angle and listening condition. Behavioral measures included detection and reaction time obtained from a sound localization task as well as K-SPIN test.
Results: Behavioral results revealed that UD group had lower performance for detection in sound localization compared to NH. In addition, the percent correct for 60^o in quiet was higher than -15^o, center, and all angles in noise condition. Electrophysiology data showed that N1/P2 amplitudes in quiet were significantly larger compared to noise condition. To examine the side of deafness on cortical response, we compared Rt. and Lt. deafness, and results showed that the N1/P2 amplitudes in Rt. deafness were larger than Lt. deafness. Dipole source analysis revealed significant noise and group effects such that N1/P2 dipole amplitudes were greater in quiet compared to noise condition, and the N1 dipole amplitudes in Lt. deafness were larger than Rt. deafness and NH groups. Hemispheric asymmetry analysis revealed that P2 dipole activity was greater in contralateral to the stimulated ear during quiet listening, but the hemispheric asymmetry weakened with noise masking. Correlations between scalp-recorded N1/P2 amplitudes and angle of sound were associated with sentence recognition scores (with N1 LFC: $r = -0.7$, with P2 RFC: $r= -0.64$).
Conclusions: The effects of noise masking on sound localization can be reflected in cortical responses in UD patients. Hemispheric asymmetry results suggest that N1/P2 measures to sound location with noise masking may distinct depending on the side of deafness. Additionally, the sensor level N1/P2 appears to have a better overall relationship to speech perception in UD compared to source activity.

Disclosures: **J. Lee:** None. **J. Han:** None. **H. Lee:** None.

Poster

222. Auditory Processing

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Topic: D.06. Auditory & Vestibular Systems

Support: NIH grant R01 DC005775

Title: Chronic bilateral stimulation through cochlear implants during development can reverse the effect of early-onset deafness on neural ITD sensitivity

Authors: W. SUNWOO, B. DELGUTTE, ***Y. CHUNG**;
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Abstract: Bilateral cochlear implant (CI) users with a pre-lingual onset of hearing loss show poor sensitivity to interaural time differences (ITD) compared to those with post-lingual hearing loss. Similarly, neural ITD sensitivity in the inferior colliculus (IC) of rabbits that are deafened as neonates is degraded compared to animals deafened as adults. Here we investigated whether chronic bilateral CI stimulation during development can reverse the effect of early-onset deafness on ITD sensitivity. Four Dutch-belted rabbits were deafened as neonates with daily injection of neomycin and then bilaterally implanted at 2 months of age. Starting just after implantation, they received daily stimulation (5 hrs/day) by environmental sounds using wearable sound processors programmed with the "Fundamental Asynchronous Stimulus Timing" (FAST) strategy designed to deliver ITD information effectively with bilateral CIs. Single-unit recording from the IC using an unanesthetized preparation commenced at 5 months of age. Stimuli were periodic trains of biphasic electric pulses with varying pulse rates (20 - 640 pps) and ITDs (-2000 to +2000 μs). The results are compared to measurements from adult-deafened rabbits (Chung et al., J Neurosci. 36:5520) and early-deafened rabbits that did not receive daily stimulation (Chung et al. JARO. 20:37). More IC neurons in the stimulated rabbits showed significant ITD sensitivity in their overall firing rate (75%) compared to unstimulated animals (62%). The difference in prevalence of ITD sensitivity was most prominent at high pulse rates (>200 pps). ITD sensitivity based on analysis of variance and neural ITD discrimination thresholds also showed improvements in the stimulated animals compared to unstimulated animals, with the largest effect found at high pulse rates. The fraction of ITD sensitive neurons, and ITD STVRs and thresholds in the stimulated animals were comparable to those from adult-deafened animals at high pulse rates. In summary, chronic bilateral cochlear implant stimulation during development can partly reverse the degradation in neural ITD sensitivity resulting from early-onset deafness. The effect is most pronounced in response to high-rate stimulation.

Disclosures: **W. Sunwoo:** None. **B. Delgutte:** None. **Y. Chung:** None.

Poster

222. Auditory Processing

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Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant DC016054
NIH Grant DC13074
NIH Grant DC016324

Title: Enhancement of spontaneous glutamate release by group I mGluRs in the medial nucleus of the trapezoid body

Authors: K. PENG[1], X. WANG[2], Y. WANG[3], D. LI[4], H. HUANG[4], *Y. LU[1];
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Abstract: Neuromodulation mediated by metabotropic glutamate receptors (mGluRs) regulates many brain functions, however, the modulatory roles of mGluRs in auditory processing are not well understood. The medial nucleus of the trapezoid body (MNTB) is a critical nucleus in the auditory brainstem circuits involved in sound localization. MNTB neurons are excited by glutamatergic inputs from bushy cells in the contralateral anteroventral cochlear nucleus (AVCN) via the giant calyx of Held synapse. MNTB neurons also receive inhibitory inputs mediated by GABA and glycine. The integration of these synaptic inputs determines the MNTB's output. Our recent study shows that group I mGluRs (mGluR I) exert neurotransmitter- and release-mode-specific modulation on the inhibitory transmission in MNTB. Here, we further investigated the modulatory effects of mGluR I on the excitatory transmission, using whole-cell recordings from brainstem slices obtained from P12-P22 (postsynaptic recordings) and P8-P10 (calyx recordings) mice. Activation of mGluR I by 3,5-DHPG (200 μM) produced an inward current, and increased glutamatergic sEPSC frequency and amplitude in MNTB neurons. Accordingly, under current-clamp configuration, 3,5- DHPG depolarized MNTB neurons, increased sEPSP frequency and amplitude, and in some cells produced action potentials, which persisted after synaptic receptors were blocked. In AVCN bushy cells, after blocking the known synaptic receptors with a cocktail of blockers (APV, 50 μM; DNQX, 50 μM; gabazine, 10 μM; strychnine,1 μM), 3,5-DHPG (200 μM) depolarized the membrane without generating action potentials. The effect on sEPSCs was blocked by a voltage-gated sodium channel (Na_v) antagonist (tetrodotoxin, 1 μM). Presynaptic (calyx) recording showed that 3,5-DHPG shifted the persistent Na^+ currents (I_{NaP}) activation to more hyperpolarized voltages and increased the I_{NaP} at the voltages around resting membrane potentials. Blockade of voltage-gated calcium channels (Ca_v) or IP_3Rs, partially eliminated the modulatory effects. Immunolabeling indicated a presynaptic expression of mGluR5. Our data indicated that activation of mGluR I increases spontaneous glutamate release and cellular excitability, affecting the output of MNTB neurons.

Disclosures: K. Peng: None. **X. Wang:** None. **Y. Wang:** None. **D. Li:** None. **H. Huang:** None. **Y. Lu:** None.

Poster

222. Auditory Processing

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Topic: F.01. Neuroethology

Support: ONR N00014-17-1-2736

AFOSR FA9550-14-1-0398
NSF NCS-FO 1734744

Title: Two-photon calcium imaging of auditory processing in the echolocating bat

Authors: M. J. WOHLGEMUTH, III[1], *J. LAWLOR[1], C. F. MOSS[1,2,3], K. V. KUCHIBHOTLA[1,2];
[1]Psychological and Brain Sci., [2]Dept. of Neurosci., [3]Dept. of Mechanical Engin., Johns Hopkins Univ., Baltimore, MD

Abstract: As humans and animals operate in the natural world, they receive, process, and react to a wide variety of complex stimuli. To investigate how the brain sorts and differentially responds to environmental stimuli, we exploit an animal model long-studied for its active sensing in the natural world: the echolocating bat. The echolocating bat produces sonar vocalizations and listens to returning echoes to determine the identity and location of objects in the environment. The bat also modifies its vocalizations to extract different features of its acoustic scene, with specific adaptations in response to an object's distance, motion, or the presence of background clutter. The spectro-temporal changes in vocal parameters correspond to specific stimulus configurations in the bat's auditory scene and provide explicit information about the signals used to guide orienting behaviors. Central to sensorimotor integration is the midbrain superior colliculus (SC). The SC is a laminated structure, with dorsal layers primarily responsive to the location of objects in egocentric space, and ventral layers involved in generating orienting behaviors to environmental stimuli. In the bat SC, neurons in dorsal layers are selective to spectro-temporal features of sonar sounds and neurons in more ventral layers respond to a wider range of acoustic stimuli. We hypothesize that laminar differences in SC stimulus response selectivity are driven by network interactions of neighboring neurons. Traditional multi-channel electrophysiology techniques can only partially assay network level activity, and as such, we are establishing methods of two-photon calcium imaging in the echolocating big brown bat, *Eptesicus fuscus*, to assay population-level activity. We first tested several different adeno-associated virus (AAV) serotypes, as well as the efficacy of a variety of cell-specific promotors. Optimized AAV techniques to drive the expression of GCaMP6s are now being employed to assay the network activation of SC neurons in response to natural echolocation sounds, as well as pure tones and noise bursts. With these population data, we will analyze networks driving stimulus selectivity in complex, natural scenes.

Disclosures: M.J. Wohlgemuth: None. **J. Lawlor:** None. **C.F. Moss:** None. **K.V. Kuchibhotla:** None.

Poster

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ONR (CM): N00014-17-1-2736
AFOSR (CM): FA9550-14-1-0398
NSF (CM): NCS-FO 1734744

Title: Evidence of in-flight head stabilization behavior in the Egyptian fruit bat: Association with wingbeat cycle and vocal emissions

Authors: J. ROSSBOROUGH[1], L. STIDSHOLT[2], A. SALLES[3], P. MADSEN[2], C. F. MOSS[3], ***L. F. HOFFMAN**[1];
[1]Head & Neck Surgery, Geffen Sch. of Med., Los Angeles, CA; [2]Zoophysiology, Dept. of Biosci., Aarhus Univ., Aarhus, Denmark; [3]Johns Hopkins Univ., Baltimore, MD

Abstract: A critical component of animal navigation behavior is the stabilization of sensory cues relative to head- or body-centric reference frames, particularly during locomotion. This is especially challenging for animals endowed with capabilities for flight where body kinematics oscillate with wingbeat frequencies that can exceed 15 Hz (Norberg & Norberg 2012). Recent evidence indicates that vision plays a prominent role for in-flight sensory stabilization in birds (Kress et al. 2015), to which the vestibular labyrinth likely enhances visual gaze stabilization through the vestibulo-ocular reflex. Even greater challenges are experienced by bats navigating in complete darkness, whereby echolocation is utilized to probe the environment and contribute to acoustic gaze. However, inflight head kinematics in bats have not been rigorously explored, and evidence for which would be the first step in exploring mechanisms of head stabilization and integration with echolocation. We investigated inflight head and body kinematics from two Egyptian fruit bats (*Rousettus aegyptiacus*) by securing "motion tags" on the head and back (i.e. between the wings). These hardware platforms included 3-axis accelerometer packages and ultrasonic microphones to record sonar tongue clicks emitted during flight. Accelerometer axes of the head tag corresponded to naso-occipital, interaural, and dorsoventral axes (i.e. G_{xH}, G_{yH}, and G_{zH}); the axes of the body tag were and spinal, left-right, and dorsoventral axes for the body tag (i.e. G_{xB}, G_{yB}, and G_{zB}). Both subjects exhibited similarity in their inflight G_{zB} profiles, exhibiting peak-peak oscillations >50m•s^{-2} corresponding to wingbeats. Negative G_{zB} accelerations corresponded to Earthward movements and upbeat of the wings. These exhibited

mean periods of approximately 0.12s, corresponding to wingbeat frequencies of approx. 8Hz. G_{zH} was remarkably similar in magnitude and period as *negative* G_{zB}. However, $+G_{zH}$ was strikingly different than $+G_{zB}$ (corresponding to wing downbeat). These data indicate that head kinematics are uncoupled from body kinematics during wing downbeat ($+G_z$). The time of the first click of click pairs was analyzed during the middle 67% of each flight epoch. The magnitude of G_{zH} was determined at the time of each first click to investigate correlates of the wingbeat cycle to the time of the first click emission. For each animal >400 clicks were analyzed, the vast majority of which occurred during positive G_{zH}. These data indicate that most of the acoustic probing occurred during when head stabilization behaviors are employed, similar to behaviors exhibited by birds to optimize visual gaze stabilization.

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Poster

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Topic: F.01. Neuroethology

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AFOSR (FA9550-14-1-0398NIFTI)
ONR (N00014-17-1-2736)

Title: Prediction strategies for target tracking in the echolocating bat, Eptesicus fuscus

Authors: *A. SALLES, C. DIEBOLD, C. F. MOSS;
Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: Tracking behaviors in real-world scenarios require fast processing of sensory information to guide adaptive motor commands. Thus, the brains of many animals have evolved to implement object motion prediction to enable target tracking and interception. Echolocating bats use echoes from their sonar vocalizations to represent the position of objects, including moving insect prey. The brains of these mammals must compute the spatial location of objects carried by a series of acoustic snapshots and use this information to track targets in a dynamic auditory scene. We hypothesize that bats, like many other aerial hawking predators, build prediction models to anticipate future locations of targets moving in time and space. Also, we hypothesize that they adjust their prediction models in response to dynamic echo information from moving prey. We trained big brown bats, *Eptesicus fuscus*, to track a target moving across their acoustic field while perched on a platform. During test sessions, several conditions were run

to probe the bat's tracking behavior and prediction models of target trajectories. This was done by changing the speed of the target or occluding the target for segments of the trajectory, or combinations of these conditions. We tracked head movements and sonar vocalizations to assess the strategies utilized by the bat to track the moving prey. Our results show that *Eptesicus fuscus* move their heads to where the target will be before the target reaches that position. Furthermore, bats continue to show anticipatory head directing behavior even when the target is partially occluded along its trajectory. Data also reveal that bats adjust their sonar behavior when their prediction of target trajectory is violated by a sudden change in target velocity during the occlusion phase of a trial. This research shows that echolocating big brown bats, *Eptesicus fuscus*, are able build prediction models to track the trajectory of a moving target.

Disclosures: A. Salles: None. **C. Diebold:** None. **C.F. Moss:** None.

Poster

222. Auditory Processing

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Program #/Poster #: 222.17/J43

Topic: D.09. Multisensory Integration

Support: NS39460 (MAM)
CIHR (SGL)
CFI (SGL)

Title: Comparison of dendritic spine density/size for anterior auditory field neurons from early-deaf and hearing cats

Authors: *M. A. MEREDITH[1], S. G. LOMBER[2], H. CLEMO[1];
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Abstract: Following early deafness, the Anterior Auditory Field (AAF) is crossmodally reorganized to exhibit visual/somatosensory activity. Unlike other auditory fields following deafness, the AAF incurs a substantial loss of inputs from other auditory cortices (Wong et al 2015). This and other functional differences among auditory regions in deaf cats suggests that the AAF may exhibit area-specific distinctions in synaptic plasticity following deafness. The present study measured dendritic spine features of spine density and spine head diameter from AAF neurons in early-deaf cats (D; ototoxin given within first postnatal month; confirmed by flat ABR) and hearing (H) controls. After reaching adulthood, the AAF (D=3; H=3) was incubated for Golgi-Cox staining. Reactive AAF neurons were visualized and their dendritic spine features assessed using light microscopy controlled by Neurolucida software. The overall spine density (809 dendritic segments) did not vary significantly (H=0.76 spines/um +/- 0.001 se; D=0.78

spines/um +/- 0.01). However, spine density significantly increased in the granular (thalamo-recipient) layer (H=0.55 spines/um +/1 0.02; D=0.71 spines/um +/- 0.02; p<0.001) but was not changed in the supra- or infragranular layers. Measures of dendritic spine head diameter (n=9011) revealed that spine heads from early-deaf animals were significantly larger (D=0.59 um +/- 0.002) than those of their hearing counterparts (H=0.53 um +/- 0.002; p<0.0001), and this effect was seen across all layers. These data indicate that dendritic spines in AAF react to early hearing loss in a lamina-dependent manner. Because the supragranular layers incur a substantal loss of inputs from other auditory cortices (such as A1) after deafness, it is provocative that the granular (thalamo-recipient) layers of AAF actually show an increase in spine density while no change in spine density was observed in the supragranular (corticocortical-recipient) layers. In summary, when compared with other auditory areas following deafness, these results reaffirm the notion that cortical crossmodal plasticity employs different synaptic strategies for different regions.

Disclosures: **M.A. Meredith:** None. **S.G. Lomber:** None. **H. Clemo:** None.

Poster

222. Auditory Processing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 222.18/J44

Topic: D.06. Auditory & Vestibular Systems

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Whitehall foundation grant

Title: Spectral cues are essential for the auditory azimuthal topographic map in the mouse superior colliculus

Authors: *S. ITO[1], Y. SI[2], D. FELDHEIM[2], A. M. LITKE[1];
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Abstract: Sound localization is a consequence of a sophisticated analysis of vibrations detected by an animal's ears. Unlike vision or touch where the receptor position of the sensory detector encodes spatial information, the incident direction of the sound source must be computed. This calculation is based on three cues: interaural timing differences (ITDs), interaural level differences (ILDs) and the spectral modification of the sound as it enters the ear (spectral cues). Traditionally, ITDs and ILDs have been considered as the two primary cues for sound localization in the horizontal plane. Spectral cues are hypothesized to be used for resolving the

front-back ambiguity and for determining the sound source elevation. However, the contribution of spectral cues in azimuthal sound localization has not been evaluated quantitatively. To measure this contribution, we conducted an electrophysiological study in the mouse superior colliculus (SC) using a 256-channel 4-shank silicon probe recording system. The SC is the best brain area to find spatially tuned auditory neurons that form a topographic map of auditory space. We used the head-related transfer functions (HRTFs)—acoustic modulations by a head and pinnae—to present virtual auditory space stimuli to an alert head-fixed mouse while recording from neurons in the SC. This allowed us to measure the receptive field (RF) properties of the neurons, determine the topographic properties of the auditory spatial map and measure the relative contributions of the ITDs, ILDs, and spectral cues. We: (1) measured, for the first time, a topographic map of auditory space in the mouse SC; (2) quantified the relative importance of the sound localization cues; (3) found that the use of the cues is heterogeneous across the SC; and (4) found that spectral cues are essential for neurons' RFs and the azimuthal topographic map in the mouse SC. These results are important for resolving the current controversy of the role of spectral cues and demonstrate how mice exploit available information to determine a sound source direction.

Disclosures: S. Ito: None. **Y. Si:** None. **D. Feldheim:** None. **A.M. Litke:** None.

Poster

222. Auditory Processing

Location: Hall A

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Program #/Poster #: 222.19/J45

Topic: A.08. Development of Motor/ Sensory/ and Limbic Systems

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Title: Age-related differences in auditory ERP responses to sounds of varying loudness in autism and typical development

Authors: *P. DWYER[1], R. DE MEO-MONTEIL[2], C. D. SARON[2], S. M. RIVERA[1];
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Abstract: Introduction: Unusual sensory processing patterns in autism spectrum development (ASD) are related to auditory ERPs (Donkers et al., 2015). However, little is known about age-

related changes in sensory ERPs in ASD. Furthermore, additional research is required, even in typical development (TD), to understand how stimulus properties such as loudness affect auditory ERPs. The present study, based on a large sample of young children from the Autism Phenome Project, aims to explore age-related differences in ERPs to sounds of differing loudness in ASD and TD.

Methods: Participants with usable ERPs were 130 children with ASD (110 male, M_{Age} = 38.50mos, SD_{Age} = 6.02mos, M_{DQ} = 65.82, SD_{DQ} = 21.23) and 81 children with TD (52 male, M_{Age} = 37.09mos, SD_{Age} = 6.46mos, M_{DQ} = 107.37, SD_{DQ} = 11.48). While watching a quiet video, participants heard, via headphones, brief tones randomly varying in loudness between 50, 60, 70 and 80 dB SPL (~200-300 trials/intensity) at an ISI randomly varying between 1-2s. 61-channel EEG was sampled at 1000 Hz. The global field power (GFP) was normalized by dividing GFP in each condition by the average across conditions, yielding an index of the relative strength of the brain's response in each condition. Correlations between age and normalized GFP were examined from 1 - 350ms using a permutation test (Maris & Oostenveld, 2007). Correlations between age and the latency of the peak of the brain's main auditory response (P1) in the raw GFP were also investigated.

Results: In TD, the relative strength of the response to 50 dB sounds was negatively associated with age from 243 - 319 ms, $p = 0.005$, such that older participants had weaker responses to soft sounds. In ASD, older children also exhibited weaker responses to soft sounds, $p = 0.021$, but this effect was observed from 176 - 201 ms. During each of these time windows, there were minimal or no correlations between the 50dB response and age in the other group. Correlations between age and responses in other loudness conditions were not significant.

Peak latency to 50 dB tones was negatively associated with age in ASD, $r = -.36$, $p = .002$, but this was not significant in TD, $r = -.15$, $p = .28$. Latency to 60 dB tones was negatively associated with age in TD, $r = -.26$, $p < .05$, but not significantly in ASD, $r = -.11$, $p = .34$. Correlations were not significant in 70 dB or 80 dB.

These results suggest the existence of a normative developmental shift in the relative amplitudes of brain responses to soft sounds in the range of 50 dB. Additional research is required to understand the mechanism of this shift, but it could reflect tuning of cortical inhibition. Furthermore, the results of the present study suggest that this shift unfolds atypically in ASD.

Disclosures: P. Dwyer: None. **R. De Meo-Monteil:** None. **C.D. Saron:** None. **S.M. Rivera:** None.

Poster

222. Auditory Processing

Location: Hall A

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Program #/Poster #: 222.20/J46

Topic: A.08. Development of Motor/ Sensory/ and Limbic Systems

Support: Fondation de France "Neuro-développement et autisme 2018"
ANR-09-MNPS-006-01, DELTA_BRUSH

Title: Source localization of auditory-evoked potentials in preterm human neonates

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Abstract: Introduction: Localization of electro-encephalographic (EEG) generators in preterm newborns rises methodological issues because of the lack of adequate head and brain models, with particular regard to cortical size, shape and electrical field propagation which change dramatically throughout development. Here, we built realistic head models from individual structural magnetic resonance images (MRI) to reliably estimate cortical generators of auditory evoked potentials (AEPs) in preterm newborns aged between 30 and 38 postmenstrual weeks (PMW). **Methods:** Realistic conductivity head-models, comprising scalp, skull and intra-cranial layers, were built from structural MRIs of 3 newborns without neurological risk, aged 33.7, 35, and 38.4 PMW respectively. 32-channel EEG recordings were performed on 30 other healthy preterm neonates aged between 30 and 38 PMW while auditory “click” stimuli were presented binaurally to each neonate (mean number of analyzable trials after artifact rejection was 41 +/- 35 per recording). Electrodes were digitized on a subject-specific molded head and electrode positions were registered to one of the 3 conductivity models according to age. A lead-field matrix relating source activations to potentials at electrode sites was computed for a source distribution constrained to the cortical surface of the head model, with source orientations normal to the surface. Simultaneous amplitude time-courses in a 2-second post-stimulus-onset window were estimated for the entire source distribution using weighted minimum norm estimation (wMNE), with a 2-second pre-stimulus window as baseline for noise covariance estimation. **Results:** Topographies of grand-averaged source amplitude time-courses revealed wide-spread activations in bilateral temporal lobes, whatever the newborn’s age. Earliest activations peaked around 250 ms after stimulus onset on the anterior part of the superior temporal gyrus, followed by more posterior activations spread on the superior and middle temporal gyri between 300 ms and 900 ms post-stimulus onset. **Conclusion:** Precise location and actual spread of source activations are, here as in general, difficult to estimate from EEG data. However, combination of our realistic head-models with wMNE yields source estimates that are compatible with preterm AEPs originating from bilateral primary and secondary auditory cortices.

Disclosures: D. Arzounian: None. **V. Delattre:** None. **J. Dubois:** None. **L. Hertz-Pannier:** None. **C. Chiron:** None. **F. Wendling:** None. **M. Hassan:** None. **A. Kaminska:** None.

Poster

223. Auditory Processing: Perception, Cognition, and Action

Location: Hall A

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Program #/Poster #: 223.01/K1

Topic: D.06. Auditory & Vestibular Systems

Support: NIH/NIDCD 5R01DC000100-40

Title: A cortical network model for solving the cocktail party problem using spatial attention

Authors: *K. F. CHOU, M. SHIREY, E. A. ROBERTS, H. COLBURN, K. K. SEN; Boston Univ., Boston, MA

Abstract: The human brain is an astonishingly powerful computational device, capable of feats yet to be matched by machines. One impressive example is the brain's ability to solve the *cocktail party problem*. At a crowded cocktail party, a normal hearing listener can attend to a friend and hear what they are saying in the midst of multiple simultaneous speakers. In stark contrast, this problem remains highly challenging for humans with impaired hearing and for machines. The complex, integrative nature of the cocktail party problem, and previous studies, suggest that auditory cortex plays an important role in solving this problem. At a cocktail party, a listener can *monitor* the entire auditory scene to detect potential targets, *select* a target at a particular location, and *switch* to another target at a different location. Thus, top-down control of cortical circuits for *flexible spatial processing* is thought to be critical for solving the cocktail party problem. Previous computational approaches to the cocktail party problem have largely focused on modeling bottom-up stimulus-driven spatial processing, and a computational framework for top-down attentional control of cortical spatial processing circuits is currently lacking. Such a framework can motivate novel experiments and brain-inspired algorithms. Here, we present a novel cortical network model that employs attentional inhibitory modulation (AIM) to solve the cocktail party problem. Specifically, AIM uses top-down attentional modulation of distinct populations of cortical inhibitory neurons to control bottom-up, cortical, spatial-processing circuits. We demonstrate that this mechanism enables the network to broadly monitor the auditory scene, aim the spotlight of attention to select a target sound, and switch to a different target, in a flexible and dynamic manner. We use AIM to identify experimentally testable hypotheses on cortical mechanisms, and to develop a brain inspired algorithm for solving the cocktail party problem incorporating spatial attention.

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Poster

223. Auditory Processing: Perception, Cognition, and Action

Location: Hall A

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Program #/Poster #: 223.02/K2

Topic: D.06. Auditory & Vestibular Systems

Support: a grant from KIRAMS
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Korea Ministry of health and welfare HO15C0003

Title: Pet and MR evidence of cognitive deficit after hearing loss in 5xFAD mice

Authors: J. KIM[1,3], ***M.-H. PARK**[4,5], H.-J. LEE[2], S. LEE[1], H. LEE[5], Y. JEONG[2], Y. SON[2], J. KIM[6], Y. LEE[1];
[1]Div. of RI application, [2]Div. of Radiation Biomed. Res., KIRAMS, SEOUL, Korea, Republic of; [3]Radiological and Medico-Oncological Sciences, Univ. of Sci. and Technol., Seoul, Korea, Republic of; [4]Otorhinolaryngology, Boramae Med. Center, Seoul Metropolitan Governm, Seoul, Korea, Republic of; [5]Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of; [6]Bio-Convergence Engin., Korea Univ., Seoul, Korea, Republic of

Abstract: The study of cognitive impairment associated with hearing loss has recently garnered considerable interest. However, the relationship between them has not been directly investigated. We used positron emission tomography (PET) and magnetic resonance imaging (MRI) to evaluate changes in glucose metabolism and gray matter concentrations in the 5xFAD Alzheimer's Disease transgenic mouse model with (AD-HL) and without (AD) hearing loss.
We found lower cerebral glucose metabolism in the frontal association cortex in the AD-HL group than in the AD group at 3 and 7 months following induction of hearing loss. While we found lower glucose metabolism in the hippocampus and cerebellum in the AD-HL group than in the AD group at 3 months, gray matter concentrations in these regions were not significantly different between the groups. Further, gray matter concentrations in the simple lobule, cingulate/retrosplenial cortex, substantia nigra, retroethmoid nucleus, medial geniculate nucleus, and anterior pretectal nucleus at 7 months were significantly lower in the AD-HL group than in the AD group. Behavioral data from the Y-maze and passive avoidance tests revealed greater memory deficits in the AD-HL group than in the AD group.
Together, these results indicate that even partial hearing loss can aggravate cognitive impairment in Alzheimer’s Disease.

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Poster

223. Auditory Processing: Perception, Cognition, and Action

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

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Topic: D.06. Auditory & Vestibular Systems

Support: Wellcome Trust Senior Clinical Fellowship (WT106964MA)
Wellcome Investigator Award (WT092606AIA)
Wellcome Trust Programme grant (WT093104)

Title: Neural correlates of figure-ground segregation in anterolateral fields of the monkey auditory cortex

Authors: *F. SCHNEIDER, F. BALEZEAU, Y. KIKUCHI, C. I. PETKOV, A. THIELE, T. D. GRIFFITHS;
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Abstract: Across sensory modalities, figure-ground segregation is critical for scene analysis. This is a particularly challenging problem in the auditory system where different sound objects emanating from the same spatial location have to be dynamically decoded using spectro-temporal features that are difficult to segregate from noisy backgrounds. Previous imaging studies have shown an involvement of non-primary auditory regions in both humans (Teki et al. 2011, 2016) and macaques (Schneider et al. 2018), however, the underlying neural mechanisms remain unknown. The aim of this study was to identify the neurophysiological correlates of figure-ground segregation in the auditory cortex of macaque monkeys using fMRI-guided electrophysiological recordings.

We investigated neuronal responses to stochastic figure-ground stimuli in two macaques. We recorded spiking and local field potentials (LFPs) in the auditory core and surrounding belt while the animals performed a Go/No-Go figure detection task. We show a significant increase in firing rate to auditory figures across cortical areas, with shorter response latencies in the anterior compared to the posterior recording regions. A figure modulation index revealed a comparable effect size across fields but we found a higher fraction of figure-ground responsive cells in the anterolateral auditory cortex. Generally, higher figure coherence causes earlier and larger increments in firing rate. The analysis of LFPs revealed figure-ground related changes in the beta and gamma band.

Our results indicate that this form of auditory scene analysis depends on anterolateral auditory fields and suggest that bottom-up information is first integrated in subpopulations of neurons further along the ventral processing pathway in the auditory cortical hierarchy. These neurons respond to a broad range of frequency bands and can detect temporally coherent elements devoid of simple mathematical relationships between acoustical components. Our results confirm and

extend with direct neurophysiological evidence earlier fMRI studies (Teki et al. 2011; Schneider et al. 2018).

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Poster

223. Auditory Processing: Perception, Cognition, and Action

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 223.04/K4

Topic: D.06. Auditory & Vestibular Systems

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PAPIIT Grant IN202317
CONACyT Postdoctoral Fellowship 324946

Title: Predictive rhythmic tapping to auditory metronomes in the nonhuman primate

Authors: *Y. A. AYALA, L. PRADO, H. MERCHANT;
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Abstract: Beat entrainment is the ability to entrain one's movements to a perceived periodic sequence, such as a metronome in music. Previous studies have shown that Rhesus monkeys share some of the human capabilities for rhythmic entrainment, such as tapping regularly at the period of isochronous stimuli. Regardless, monkeys tend to tap hundreds of milliseconds after stimulus onsets and exhibit a preference for visual metronomes in contrast to humans, who tap slightly ahead of the stimulus onsets and are more sensitive in detecting and synchronizing to auditory metronomes. To test the predictive and flexibility nature of rhythmic entrainment to auditory rhythms in nonhuman primates, we trained two Rhesus monkeys to perform fast hand movements in phase to the seven stimuli of an isochronous metronome (stimulus onset intervals: 550, 650, 750, 850 ms). Monkeys were also trained to maintain their rhythmic movements when the last two stimuli of the metronome were omitted or when only one stimulus was omitted at random positions (4-6) within the metronome. First, we found that monkeys could predictively entrain to the trained and novel isochronous auditory rhythms, generating movements in anticipation of the stimulus onset, i.e., negative asynchronies. This predictive behavior also occurred when the animals synchronized their movements to metronomes with stimulus omissions at expected and unexpected positions for the largest interval (850 ms). Notably, the asynchronies at the random omitted positions were similar to the asynchronies at the same positions from trials with no stimulus omissions as also observed in humans (n=11). Second, we found that monkeys entrained to the isochronous beat using an error-correction mechanism to

compensate for prior stimulus-movement phase variability: the inter-movement interval was inversely dependent on the duration of the preceding movement. Important for archiving prediction and error correction in the monkeys' performance was to provide immediate feedback about the timing of each movement during the training period. Overall, results show that monkeys are capable of predictive and flexible entrainment to auditory rhythms even with stimulus omissions and that generalize this ability to novel untrained tempos. Our findings shift the limits of beat entrainment in nonhuman primates and advance our knowledge on the evolutionary origins of beat entrainment.

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Poster

223. Auditory Processing: Perception, Cognition, and Action

Location: Hall A

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Program #/Poster #: 223.05/K5

Topic: D.06. Auditory & Vestibular Systems

Support: EC MSC-GF 750459
RGC HK GRF 11100518

Title: Prediction error signalling in the auditory cortex: Neural omission responses in anaesthetised rats

Authors: *R. AUKSZTULEWICZ[1,2], N. S. HARPER[3], V. RAJENDRAN[1], J. W. SCHNUPP[1]; [1]Dept. of Biomed. Sci., City Univ. of Hong Kong, Hong Kong, Hong Kong; [2]Neurosci. Dept., Max Planck Inst. for Empirical Aesthetics, Frankfurt am Main, Germany; [3]Dept. of Physiology, Anat. and Genet., Univ. of Oxford, Oxford, United Kingdom

Abstract: It has proposed that at least some cortical neural responses reflect the signalling of error between predictions about stimuli and actual sensory inputs [1,2], as evidenced by work on stimulus-specific adaptation, repetition suppression, and mismatch responses [3]. However, error signals can also be studied in paradigms where an expected stimulus is omitted entirely. The resulting omission response - arguably a closer correlate of unfulfilled prediction signals than the response to a mispredicted stimulus [4] - has often been observed in non-invasive recordings in humans but has yielded limited evidence in invasive recordings in animal models [5].
We recorded local field potentials (LFP) in the auditory cortex of anaesthetised female Lister Hooded rats (N = 4) and reconstructed single- and multi-unit activity (SUA/MUA, n = 215 units total) in response to auditory stimulus omissions. Stimuli included trains of noise bursts presented at a range of fixed rates (2-4Hz) with a random subset of 5% bursts omitted. To test whether stimulus omissions result in activity reflecting either local spiking or distal inputs to the auditory cortex, we calculated MUA/SUA peri-stimulus time histograms and LFP amplitude

following stimulus omissions and compared them to activity evoked by immediately preceding acoustic bursts. To quantify low-frequency entrainment of neural activity to stimulus trains, we also calculated the amplitude and phase of slow (burst-rate) LFP components at the expected onset of omitted stimuli.
We could identify a subset of units that showed increased firing rate to omitted stimuli, relative to baseline. These omission responses had a lower amplitude and longer latency than burst-evoked sensory responses. Crucially, their latency relative to expected onset of a missing burst was not modulated by burst presentation rate, suggesting that they reflect error signalling rather than a burst-train offset response. These findings provide direct evidence for sensory prediction error signalling in auditory cortex.
[1] Singer Y, Schnupp JWH, King AJ, Harper NS, et al. (2018) eLife 7:e31557.
[2] Huang Y, Rao R (2011) Rev Cogn Sci 2:580-593.
[3] Auksztulewicz R, Friston K (2016) Cortex 80:125-40.
[4] Chennu S, Bekinschtein TA, Henson R, et al. (2016) J Neurosci 36(32):8305-16.
[5] Jongsma ML, Coenen AM, Van Rijn CM (2002) Psychophysiol 39(2):229-35.

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Poster

223. Auditory Processing: Perception, Cognition, and Action

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 223.06/K6

Topic: D.06. Auditory & Vestibular Systems

Support: 5 r01DC013961

Title: Neural correlates of auditory stream segregation in the auditory pathways of macaque monkeys

Authors: *T. BANNO[1], J. LEE[1], Y. I. FISHMAN[2], Y. E. COHEN[1];
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Abstract: A fundamental component of auditory scene analysis is grouping acoustic features from different sound sources into segregated representations of auditory objects. This process is a fundamental aspect of hearing and speech perception. Although psychoacoustic studies have shown that differences in the frequency and location of sound sources provide important cues for the perceptual segregation of auditory streams in humans, the neuronal mechanisms underlying stream segregation based on these cues remain to be fully elucidated. Here, we trained macaque monkeys to report a deviantly loud target stimulus that was embedded in one of two temporal

sequences of tone bursts; each tone burst, except a "target" tone burst had a random sound level. In humans, the detection of this target stimulus was harder when the frequency difference between the tone-burst sequences was small and became easier as the frequency difference increased. Similarly, detection became easier when the angular separation increased and harder when this separation decreased. Importantly, because the target can only be reliably detected when the two tone sequences are perceptually segregated, this stimulus paradigm provides an objective measure of auditory streaming. As in the human studies, we found that the monkeys' performance improved as the frequency difference or the angular separation of the tone-burst sequences became larger, suggesting the monkeys utilize frequency and location cues in a similar way to humans in auditory streaming. While a monkey participated in the target detection task, we recorded neuronal activity in the primary or nonprimary auditory cortex. We set the best frequency of the recorded neurons to be that of the tone-burst sequence that contained the target stimulus. We found that, in the primary auditory cortex (A1), neurons responded to each presentation of the tone bursts with the response magnitude reflecting the frequency tuning of the neurons. In contrast, neurons in the lateral belt showed sustained activity throughout the trial and the magnitude of the responses to the low- and high-frequency tone bursts was not clearly distinct, even though lateral-belt neurons had frequency tuning as sharp as those in A1. To our knowledge, this is the first study to compare neuronal responses in primary and nonprimary auditory cortex that may be relevant for auditory streaming in non-human primates. Our behavioral and physiological results suggest that this stimulus paradigm provides a promising tool for elucidating neural mechanisms contributing to auditory scene analysis.

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Poster

223. Auditory Processing: Perception, Cognition, and Action

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 223.07/K7

Topic: D.06. Auditory & Vestibular Systems

Support: NIH/NIDCD Grant R01DC016267 (GMD)

Title: Autonomic nervous system correlates of speech categorization revealed through pupillometry

Authors: *G. A. LEWIS, G. M. BIDELMAN;
Sch. of Communication Sci. and Disorders, Univ. of Memphis, Memphis, TN

Abstract: Human perception requires the many-to-one mapping between continuous elements of a physical structure and discrete sets of categorical representations. This "downsampling" operation plays a critical role in speech perception because acoustic cues do not share constant

relations with perceptual-phonetic representations. Categorical perception (CP) of speech is thought to mitigate perceptual variance by emphasizing between- rather than within-category contrasts. Beyond this benefit, CP might generate additional perceptual constancy needed to extract auditory percepts of speech from interfering sound sources (i.e., noise). Here, we used psychophysiological (pupillometry) measures to determine the degree to which noise interference impacts cognitive load and the perceptual identification of phonetic vs. non-phonetic speech features during categorization. Listeners classified a synthetic five-step acoustic-phonetic continuum of speech tokens ranging from /u/ to /a/ presented in various signal-to-noise ratios (SNRs [clear, 0 dB, -5 dB]). Continuous recordings of pupil dilation served as a measure of processing effort, with larger and later dilations reflecting increased listening demand. Critical comparisons were between time-locked changes in eye data in response to sound tokens with a clear phonetic identity (i.e. continuum endpoints, Tk1/5) vs. those without a clear phonetic identity (i.e., continuum midpoint, Tk3). Listeners' behavioral data indicated that clear speech elicited sharper psychometric functions and faster responses which steadily declined in noise. As for listeners' pupillary responses, results showed that noise increased pupil dilation across stimulus conditions, but not straightforwardly. Peak pupil size was modulated solely by SNR, being larger for degraded relative to clean speech (i.e., [0 dB and -5 dB] > clean). In contrast, peak dilation latency varied with both token and SNR. Interestingly, unambiguous tokens (Tk1/5) elicited earlier, more pronounced increases in pupil dilation relative to phonetically ambiguous speech (Tk3). Recent work has observed that pupil diameter increases for sounds participants consider more salient, vigorous, or loud, which could reflect greater listening demand or arousal. The differences in pupillary responses observed here suggest that listeners rely on perceptual categorization to reconstruct auditory percepts under challenging real-world listening conditions.

Disclosures: **G.A. Lewis:** None. **G.M. Bidelman:** None.

Poster

223. Auditory Processing: Perception, Cognition, and Action

Location: Hall A

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Topic: D.06. Auditory & Vestibular Systems

Support: NIH R90DA043849
Albstein Research Scholarship

Title: Dissociating the role of cholinergic signaling in task acquisition versus expression during sensorimotor learning

Authors: *S. ELNOZAHY[1], T. RAAM[2], A. WANG[3], S. OSTOJIC[5], K. V. KUCHIBHOTLA[4]; [1]Neurosci., [2]Psychological & Brain Sci., [4]Psychological & Brain Sciences; Neurosci., [3]Johns Hopkins Univ., Baltimore, MD; [5]Ecole Normale Superieure, Paris, France

Abstract: Performance on cognitive tasks during learning involves both the acquisition of task contingencies (e.g. stimulus-action associations), and the correct expression of that underlying knowledge in the appropriate context. Here, we use a novel behavioral paradigm (Kuchibhotla *et al*., 2019) that allows us to quantitatively dissociate knowledge acquisition from how it is demonstrably 'expressed' by changing the context of the task (presence or absence of reinforcement). We are able to dissociate the acquisition and expression of task knowledge by testing the animal in two, interleaved, contexts: a "reinforced" context, which includes reward, and a "probe" context, in which no reward is delivered. We constructed a network model to determine how stimulus-action associations can be acquired versus expressed at different rates. Our model suggests that the observed behavioral dissociation in learning occurs due to a contextual scaling factor, which biases the decision readout. Here, we test the hypothesis that optimally tuned cholinergic neuromodulation is critical for effective behavioral expression of learned knowledge.

To do so, we considered whether tonic cholinergic signaling from the basal forebrain, which projects broadly to both sensory and decision-related circuits, may play a permissive role in behavioral expression. We specifically test whether an excess of tonic cholinergic neuromodulation is responsible for suppressing task relevant information in the 'reinforced' context early in learning. We virally express eNhPR3.0 in cholinergic neurons in the basal forebrain of ChAT-cre mice . Using optogenetics, we suppress the basal forebrain in a block-based manner at a time-point early in learning where mice perform poorly in the reinforced context (e.g. lick to both target and foil tones) but at expert levels in the probe context (e.g. lick only to target tone). Preliminary experiments suggest that bilateral suppression of cholinergic activity partially "closes the gap" between performance in the probe and reinforced contexts in a rapid and reversible fashion. These data suggest that optimally tuned cholinergic neuromodulation is critical for task expression early in learning. We are monitoring activity of cholinergic axonal projections to the auditory cortex using two-photon calcium imaging to determine the tonic and phasic activity patterns of cholinergic activity during sensorimotor learning. We hope to identify the role of cholinergic signaling in modulating behavioral expression as distinct from task acquisition.

Disclosures: S. Elnozahy: None. **T. Raam:** None. **A. Wang:** None. **S. Ostojic:** None. **K.V. Kuchibhotla:** None.

Poster

223. Auditory Processing: Perception, Cognition, and Action

Location: Hall A

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Program #/Poster #: 223.09/K9

Topic: D.06. Auditory & Vestibular Systems

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NIH P01AG055365

Title: Decision making modulates neuronal responsiveness and functional connectivity in primary auditory cortex

Authors: *N. A. FRANCIS, A. SHEIKHATTAR, B. BABADI, P. KANOLD;
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Abstract: Auditory communication critically depends on our ability to recognize behaviorally meaningful sounds. We have recently shown that neurons in primary auditory cortex (A1) layer 2/3 (L2/3) form small functional networks during pure-tone detection (Francis et al. *Neuron* 2018). However, natural acoustic environments often require the listener to discriminate between target vs non-target sounds, raising the question of how the presence of non-targets affects the neural coding of task-related information in A1. Thus, we trained transgenic CBA x Thy1-GCaMP6s F1 mice to perform a go/no-go pure-tone frequency discrimination task, while we used *in vivo* 2-photon (2P) Ca^{2+} imaging during task performance to study how target and non-target sounds are encoded in A1 L2/3. The mice were trained to lick a waterspout if the pure-tone frequency was 7 or 9.9 Hz, and to withhold licking if the tone frequency was 14 or 19.8 kHz. We performed 35 experiments in 9 mice, with a total population of >2000 neurons. For each neuron, we separated responses during trials with behavioral hits, misses, false alarms, and correct rejections. By comparing neural responses to tone presentation during passive vs. behavior trials, we found that attentional gain in A1 was modulated by behavioral choice. Correct behavioral choices (i.e., hits and correct rejections) had a small negative gain. During false alarms, neuronal responses had a small positive gain, whereas a large negative gain was observed during misses. Thus, attentional gain was bidirectionally modulated by behavioral choice. Since task-related information is encoded by networks of neurons in A1, we studied functional connectivity within A1 using Granger causality (GC)—a multivariate estimate of causal 'links' between neurons. We found that decision-making modulated functional network connectivity in A1. Incorrect behavioral choices (misses and false alarms) had a higher number of GC links than correct behavioral choices (hits and correct rejections). The remaining task-dependent network changes were mainly during hit trials which, when compared to the same tone presentation during both passive and miss trials, had a significantly greater GC link weight, fewer neurons per GC subnetwork, and a greater number of GC subnetworks. Together our results suggest that discriminating between targets vs non-targets drives changes in the response amplitude and functional connectivity of neuronal populations in A1.

Disclosures: **N.A. Francis:** None. **A. Sheikhattar:** None. **B. Babadi:** None. **P. Kanold:** None.

Poster

223. Auditory Processing: Perception, Cognition, and Action

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 223.10/K10

Topic: D.06. Auditory & Vestibular Systems

Title: Cortical circuit mechanism underlying anticipatory movement

Authors: J. ZHANG[1], ***T. LI**[2], J. GUAN[1], X. LIAO[3], X. CHEN[1];
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Abstract: The ability of the brain to predict future events based on the pattern of recent sensory experience is critical for guiding animal's behavior. Neocortical circuits for ongoing processing of sensory stimuli are extensively studied, but their contributions to the anticipation of upcoming sensory stimuli remain less understood. We here used *in vivo* cellular imaging to record mouse primary auditory cortex (Au1) to elucidate its role in processing anticipated stimulation. We found neuronal ensembles in layers 2/3, 4, and 5 which were activated in relationship to anticipated sound events following rhythmic stimulation. These neuronal activities correlated with the occurrence of anticipatory motor responses in an auditory learning task. Pharmacological and optogenetic manipulation experiments demonstrate that a specific motor-related region of Au1 is required and sufficient for driving anticipatory motor responses. These results suggest that the neural circuit from the primary auditory cortex to the motor-related region is critical for coding predictive information and transforming it into anticipatory motor behavior.

Disclosures: J. Zhang: None. **T. Li:** None. **J. Guan:** None. **X. Liao:** None. **X. Chen:** None.

Poster

223. Auditory Processing: Perception, Cognition, and Action

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Program #/Poster #: 223.11/K11

Topic: D.06. Auditory & Vestibular Systems

Support: Marie-Curie Individual Fellowship (659719-AG-GF)

Title: Learning related population dynamics of the dorsal medial geniculate body in mice

Authors: *A. GILAD, I. MAOR, A. MIZRAHI;
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Abstract: Learning to associate sensory stimuli with a chosen action has been classically attributed to the cortex. Recent studies suggest that learning-related changes may already be present at the thalamus. Anatomy alone suggests that the thalamus is not just a simple sensory relay station to the cortex, but also includes attributes of higher-order processing. In the auditory system, the dorsal medial geniculate body (dMGB) projects to higher-order auditory areas and also receives feedback from the primary auditory cortex, implying that it has a role in higher-order processing. To describe the plastic changes in dMGB as a result of learning, we used time-lapse calcium imaging. We injected AAV-GCamp6f into the dMGB and implanted a 400 µm optical fiber to chronically image calcium population responses as mice learned a go/no-go auditory discrimination task. Mice gradually learned to lick to a go sound (10 kHz; 'hit') and withhold licking to the no-go sound (7.1 kHz; 'CR') while reducing their error rate ('false alarms' and 'misses') to minimum. Our dataset includes continuous recordings of dMGB responses to sounds from 8 mice - before, during and after learning. dMGB population responses were frequency specific; some recording sites showed preference to the go sound (n=5 mice) whereas others preferred the no-go sound (n=3 mice). As a result, the information contained in dMGB responses alone allows high accuracy discriminations between the go and no-go sounds; as early as 80ms after stimulus onset. Interestingly, dMGB responses in expert mice encoded the choice of the mouse, as we could rapidly (i.e. 200ms from stimulus onset) discriminate between hit and miss trials. In addition to these task-dependent signals, we observed opposite effects on the go and no-go responses. In recordings sites preferring the 'go' sounds, responses to 'go' on hit trials increased. In recordings sites preferring the 'no-go' sounds, responses to 'no-go' sounds decreased. There was a strong correlation between the time in which the mouse crossed behavioral threshold to expert level and the time in which dMGB responses displayed the strongest modulation (either go enhancement or no-go suppression). These results show that the auditory thalamus encodes task- and learning-related information.

Disclosures: A. Gilad: None. **I. Maor:** None. **A. Mizrahi:** None.

Poster

223. Auditory Processing: Perception, Cognition, and Action

Location: Hall A

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Program #/Poster #: 223.12/K12

Topic: D.06. Auditory & Vestibular Systems

Title: Neural correlates of auditory perception

Authors: *P. KRAUSS;
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Abstract: In search of the neural correlates of auditory perception in rodents we compare three different classes of neural activity patterns recorded via a multichannel recording system: (1) stimulus driven activity, reflecting both, sensory processing and perception; (2) spontaneous activity in naïve animals, i.e. neither sensory processing nor perception; (3) activity reflecting a stable (phantom) percept without sensory processing. Therefore, we use our animal model for chronic subjective tinnitus as a tool to induce a phantom percept without sensory input. The frequency of the perceived subjective tinnitus is estimated using a well established behavioral paradigm, i.e. the gap pre-pulse inhibition of the acoustic startle reflex. We find that acoustic percepts are characterized by attractor-like spatiotemporal patterns of neuronal activity within auditory cortex. These neuronal attractors are specific for the perceptual quality of each distinguishable percept, i.e. different frequencies of acoustic stimulation or silence, respectively. In case of subjective tinnitus, the neural attractor that can be measured during silence is shifted into that location where the corresponding stimulus driven activity leading to a similar perceptual quality as the tinnitus is represented.

Disclosures: P. Krauss: None.

Poster

223. Auditory Processing: Perception, Cognition, and Action

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Topic: D.06. Auditory & Vestibular Systems

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P50MH109429

Title: The role of saccadic vs. environmental visual rhythms in regulating auditory cortical excitability

Authors: *M. N. O'CONNELL, A. BARCZAK, T. MCGINNIS, S. NEYMOTIN, P. LAKATOS;
Ctr. for Biomed. Imaging and Neuromodulation, Nathan S Kline Inst., Orangeburg, NY

Abstract: One of the ways we perceive our external world is through the process of “active sensing” in which biological sensors (e.g. fingers and eyes) sample the environment utilizing mostly rhythmic motor routines. Several previous studies indicate that these “motor sampling patterns” modulate neuronal excitability in sensory brain regions by entraining brain rhythms, termed *active sensory entrainment*. On the other hand, brain rhythms can also be entrained by rhythms of the external environment that are independent of internal motor commands (sensory entrainment or *environmental entrainment*), which is most common in the auditory modality.

The goal of our study was twofold. First, we wanted to investigate the properties of *active sensory entrainment* in the auditory system, using the most prominent motor sampling pattern in primates: saccades (eye movements). Second, we wanted to explore how/whether active sensory entrainment by saccades interacts with *environmental entrainment* by visual stimuli.
Neuroelectric activity was recorded using linear array multielectrodes in the primary auditory cortex (A1) of awake macaques during two conditions: 1) in the absence of any stimuli (resting state condition), and 2) during the presentation of a rhythmic (1.8Hz) stream of LED flashes (visual stimulation condition). Eye position was continuously monitored.
In the resting sate condition, we found that cortical excitability of A1, as indexed by multiunit activity (MUA) and current source density (CSD), was indeed entrained by saccades. We also verified that during visual stimulation, neuronal excitability in A1 was entrained by the rhythmic LED flashes. Strikingly, while LED flashes entrained oscillations to their high excitability phases, saccades entrained them to their low excitability phases in A1. We also found that during the visual stimulation condition, oscillations were either entrained by the LED flashes or saccades but never both.
Taken together, our results indicate that when attention is guided by environmental visual rhythms, like the LED flashes (environmental entrainment), temporally synchronized auditory inputs would be enhanced, while during entrainment by saccades (active sensory entrainment), temporally synchronized auditory inputs would become suppressed.

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Poster

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Program #/Poster #: 223.14/K14

Topic: D.06. Auditory & Vestibular Systems

Support: Uehara Memorial Foundation

Title: Neural encoding and decoding of auditory cortex during perceptual decision making

Authors: *A. FUNAMIZU[1], F. MARBACH[1], A. M. ZADOR[2];
[1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [2]Zador Lab., Cold Spring Harbor Lab., Cold Spg Hbr, NY

Abstract: Neurons in auditory cortex encode auditory stimuli, but the precise encoding can depend strongly on task-relevant variables such as stimulus or reward expectation. This raises the question: If the cortical representation of the stimulus varies with task-relevant variables, how can areas downstream of auditory cortex decode these representations? One possibility is that

decoding in downstream areas also depends on these task-relevant variables. To address this question, we developed a two-alternative choice auditory task for head-fixed mice in which we varied either reward expectation (by varying the amount of reward, in blocks) or stimulus expectation (by varying the probability of different stimuli). We then used calcium imaging to record populations of neurons in auditory cortex while mice performed the task. We found that varying either reward or stimulus expectation changed neural representations (i.e. stimulus encoding), sometimes dramatically. However, the optimal decoder was remarkably invariant to different encodings induced by different expectations. Our results suggest that stimuli encoded by auditory cortex can be reliably read out by downstream areas, even when the encoding is modulated by task-relevant contingencies.

Disclosures: **A. Funamizu:** None. **A.M. Zador:** None. **F. Marbach:** None.

Poster

223. Auditory Processing: Perception, Cognition, and Action

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Title: Exploring the effect of pre-trial population activity in the auditory cortex on trial outcome during delayed frequency discrimination in mice

Authors: *D. REATO, R. STEINFELD, A. TACAO-MONTEIRO, A. RENART;
Champalimaud Ctr. for the Unknown, Lisbon, Portugal

Abstract: Fluctuations in neural activity not locked to external events or motor responses are referred to as ‘spontaneous activity’. A major organizational feature of spontaneous activity are varying degrees of overall synchronization among groups of nearby neurons. According to this measure, cortical circuits can find themselves in ‘synchronized’ or ‘inactivated’ states, in which neurons are globally synchronized on time-scales of hundreds of milliseconds - leading to large magnitude slow oscillations in population firing rate, or in ‘desynchronized’ or ‘activated’ states, in which such slow fluctuations are largely absent, population activity is tonic, and population-averaged spiking correlations are weak. The degree of synchronization across local circuits can vary with fast time-scales, comparable to the duration of trials in psychophysical tasks. We explored the relationship between baseline population activity in the mouse auditory cortex, and

the outcome of a delayed response (delay period of 0.5 sec) frequency discrimination task on a trial-by-trial basis. Whereas previous studies have shown a relationship between baseline activity and trial outcome in auditory detection - indicating an effect of brain state on the tendency of mice to respond - the effect of brain state on discrimination accuracy - which should be independent of responsivity - is not yet clear. We assessed the effect of baseline firing rate and degree of synchronization on accuracy and on the tendency of mice to 'skip' a response after the delay period. We find a trend towards a higher probability of skipping a response as the circuit becomes more desynchronized and a non-monotonic relationship between population firing rate (normalized to the average in the session) and skips, with a minimum for firing rates close to the average. On the other hand, both firing rates and the degree of synchronization lack a clear relationship with accuracy. These findings are in contrast to previous results from our laboratory and others on the effect of synchronization on the representation of sounds in anesthetized preparations.

Disclosures: D. Reato: None. **R. Steinfeld:** None. **A. Tacao-Monteiro:** None. **A. Renart:** None.

Poster

223. Auditory Processing: Perception, Cognition, and Action

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Topic: D.06. Auditory & Vestibular Systems

Support: NIDCD (DC05014)

Title: Neuronal signatures of task acquisition versus context-dependent expression in the auditory cortex

Authors: *C. DRIEU[1], Z. ZHU[2], S. OSTOJIC[4], K. V. KUCHIBHOTLA[3];
[1]Psychological & Brain Sci., [2]Neurosci., [3]Psychological and Brain Sciences, Neurosci., Johns Hopkins Univ., Baltimore, MD; [4]Ecole Normale Superieure, Paris, France

Abstract: Performance on cognitive tasks during learning is often used to measure knowledge, yet remains controversial since such testing is susceptible to contextual factors. Here we use a novel behavioral paradigm (Kuchibhotla *et al*., 2019) that allows us to quantitatively dissociate knowledge acquisition from how it is demonstrably 'expressed' by changing the context of the task (presence or absence of reinforcement). We have previously shown that the dynamics of knowledge acquisition versus expression could be captured using a minimal stimulus-action association model in which knowledge of the task is acquired through reward-driven reinforcement while knowledge expression is suppressed early in learning by a contextual scaling factor that operates on the decision read-out (Kuchibhotla *et al*., 2019). Here, we aim to

expand this model by adding an intermediate layer that integrates feedforward sensory inputs and then outputs to a decision readout layer; both this intermediate layer and the downstream decision layer are influenced by a contextual scaling factor. This intermediate layer is intended to model the role of the auditory cortex in reinforcement learning and will allow us to make testable predictions about how the auditory cortex may reflect computations associated with task acquisition versus expression. In parallel, we are combining our behavioral paradigm with two-photon calcium imaging in behaving mice to address how the excitatory neural network in the auditory cortex reflects sensorimotor learning, including both task acquisition and context-dependent expression. We monitor the activity of the same large population of pyramidal cells in layer II/III of the auditory cortex over the course of learning and analyze single cell and neuronal population activity profiles. Our preliminary data suggests that excitatory neural populations in the auditory cortex reflect the behavioral dissociation between acquisition and context-dependent expression, suggesting that both computations are indeed reflected at the level of the auditory cortex. Our work aims to develop a robust theoretical framework based on experimental data to identify cortical mechanisms underlying sensorimotor learning by dissociating acquisition of knowledge from its context-dependent expression.

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Poster

223. Auditory Processing: Perception, Cognition, and Action

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Title: Resolution of a perceptual decision across the layers of the auditory cortex

Authors: *R. STEINFELD, T. COSTA, D. REATO, A. TACÃO-MONTEIRO, A. RENART; Champalimaud Fndn., Lisbon, Portugal

Abstract: The functional role of the layered cortical microcircuit remains mysterious, partly because there have been relatively few investigations of the simultaneous activity of populations of neurons across layers during well controlled behavioral tasks. Here, we trained head-fixed mice to perform a 2AFC delayed response frequency discrimination task. Absence of licking during the delay period allows us to study how sensory signals are turned into categorical

choices in the absence of potentially confounding salient overt motor output. We recorded activity in the auditory cortex (AC) using 6-shank silicon probes. The probes were inserted coronally, with different shanks parallel to the cortical layers, and spanning approximately the depth of AC. Mutual information between neural activity and stimulus identity raises at stimulus onset across both mid-superficial and deep layers, and decays after stimulus offset. By the end of the 0.5 sec delay period there was barely any stimulus information left in the AC. Neurons whose activity was strongly informative about the upcoming choice, however, were only located in deep layers. Choice information was not present before stimulus onset and grew during the delay period in anticipation of the animals' responses. Importantly, the same neurons' activity preceding licks was not informative about their left-right identity when these licks occurred in the inter-trial-interval, showing that choice information is specific to the output of the auditory decision. Stimulus- and choice-informative neuronal populations in deep layers were largely non-overlapping. We are currently using functional connectivity and non-linear dynamical systems methods to assess the causal relationship between layers and between neurons with different selectivities. Our results offer a window into the distribution of labor across cortical layers in the unfolding and completion of the transformation between a sensory stimulus and the animal's internal representation of its meaning in the context of a categorization task.

Disclosures: R. Steinfeld: None. **T. Costa:** None. **A. Tacão-Monteiro:** None. **A. Renart:** None. **D. Reato:** None.

Poster

223. Auditory Processing: Perception, Cognition, and Action

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Topic: D.06. Auditory & Vestibular Systems

Support: NIMH (R00MH115082-04)

Title: Neuronal ensembles for auditory deviance detection in posterior parietal cortex

Authors: *A. B. VAN DERVEER[1], A. D. FERRELL[1], M. L. GREENE[1], J. T. HOLMES[1], V. KUBRICKA[1], J. P. HAMM[2];
[1]Neurosci. Inst., [2]Georgia State Univ., Atlanta, GA

Abstract: In the mammalian brain, neural processing of sensory events is significantly influenced by context. For instance, responses in sensory cortices are suppressed to stimuli which are repetitive or redundant with previous stimuli. However, in a context in which the same stimulus is novel, or deviates from expectations, sensory cortical responses are augmented. This contextual modulation of neural responses is a fundamental component of how the brain efficiently processes the sensory world to guide immediate and future behaviors.

Our past work has shown that in early primary sensory cortices (e.g. V1 or A1), contextual modulation is robust and is carried out by the interaction of distinct embedded neuronal subnetworks, including an ensemble of neurons which responds only to contextually "deviant" events. Given the importance of such contextual modulation for cognition and survival, we examined whether and how these computations were present in a downstream associative region known to be critical for multisensory integration and higher cognitive function: the posterior parietal cortex (PPC). With two-photon calcium imaging carried out in awake mice during a classic auditory "oddball" paradigm, we measured activity in populations of PPC neurons with single neuron resolution. Responses to redundant (88% frequency "standards") and deviant (12% frequency "oddballs") pure tone stimuli of differing pitches were compared to responses to the same pitches in a many-standards "control" context, wherein stimuli of 8 possible pitches were randomly presented.
As we have described in primary sensory regions (Hamm and Yuste, 2016; Hamm et al BioRxiv 2018), robust stimulus-specific adaptation (i.e. reduced responses to redundant) and genuine deviance detection (i.e. enhanced responses to deviants) were observed at the population level, after averaging over all neurons. Further, distinct ensembles of neurons showing stronger responses to deviant events were present, but less selective than subnetworks previously observed in upstream circuits. Instead, a proportion of neurons (roughly 40%) showed a below-baseline suppression of activity to redundant stimuli, a feature not observed in V1 or A1. Our results suggest that neuronal population's downstream regions may serve to integrate signals from separate subnetworks in basic sensory cortices, perhaps to scale and guide behaviors.

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Poster

223. Auditory Processing: Perception, Cognition, and Action

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Title: Antagonistic modulation of frequency selectivity by the amygdalocollicular and corticocollicular pathways

Authors: J. LEE[1], J. LIN[1], Z. YU[2], A. SWIERCZ[2], P. J. MARVAR[2], *G. K. WU[1];
[1]Dept. of Psychology and Inst. for Neurosci., [2]Dept. of Pharmacol. and Physiol. and Inst. for Neurosci., The George Washington Univ., Washington, DC

Abstract: Both emotion and cognition have profound effects on perception. Their synergistic actions are important to adjust sensory processing that may lead to adaptive perception for animal's communication and survival. It is largely unknown how the brain regions involving emotion and cognition work together to modulate sensory processing in subcortical sensory nuclei. The inferior colliculus, a midbrain structure in the auditory system, receives two major descending projections: one from the basal nuclei of the amygdala, and the other from the auditory cortex, which implies their response properties could be affected by emotional and cortical states. In this study, we adopted fear conditioning behavioral paradigm to elicit emotional responses of mice to acoustic cues, and performed *in vivo* recordings of the neurons in the central nucleus of the inferior colliculus to examine their frequency responses before and after fear conditioning. By pharmacologically silencing the auditory cortex and the amygdala sequentially, we teased apart the contributions of the corticocollicular and amygdalocollicular pathways: the receptive fields of neurons with their best frequency close to the conditioned stimuli were sharpened after silencing the basal nucleus of the amygdala, while they were broadened after silencing the auditory cortex. Our results showed an antagonistic modulation of frequency representation by these two pathways. It suggests that the amygdalocollicular pathway could increase the sensitivity of neurons in the inferior colliculus to detect aversive cues, while the corticocollicular pathway could increase the selectivity of these neurons to distinguish aversive cues from benign stimuli.

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Poster

223. Auditory Processing: Perception, Cognition, and Action

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Program #/Poster #: 223.20/K20

Topic: D.06. Auditory & Vestibular Systems

Title: Characteristics of the auditory cortical response of the male rat

Authors: D. PEREZ-HERNANDEZ[1], A. GALAN COLORADO[1], O. LARA-GARCIA[2], ***M. A. LARA GARCIA**[1], P. PACHECO[3];
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Abstract: Despite of an extensively literature dealing with evoked potentials responses of the auditory pathway in the cat, electrophysiological studies of auditory evoked potentials (AEPs) in the rat by auditory stimulation are poor. This lack of information results critical when ultrasonic vocalization frequencies and social behavior significance are correlated. Then, as a first step to provide an objective analyses in the present study we explore AEPs in the rat to auditory stimulation. Methods: In urethane anesthetized male Wistar rats, unilateral craniotomy to expose auditory cortex was done and using a monopolar silver chloride electrode (rounded-end) was gently place directly in the meninges. Acoustical stimulation was provided using single tone pip at 4 kHz. Results: When intensity was low, AEPs of one wave were recorded at 20 msec of latency; when sound stimulation intensity was slightly increase (medium) AEPs were increase and they were compound of two waves at 20 msec of latency, additionally at 60 msec of latency an oscillatory response was noticed at 60/sec; and when intensity was increased (high), AEPs were compound od one wave and their latency was reduced to 15 msec, also at 60 msec a longer oscillatory response was noticed. Discussion: delated oscillatory response recorded with a tone pip of 4 kHz in frequency needs to be considered when ultrasonic stimulation is used, we do not know whether ultrasonic stimulation in the rat provokes these kind of evoked responses i.e. short-latency and long-latency oscillatory responses. In preliminary observations using a single tone pip at 40 kHz, we obtained AEPs compound of long-term oscillations at 60/sec.

Disclosures: D. Perez-Hernandez: None. **A. Galan Colorado:** None. **O. Lara-Garcia:** None. **M.A. Lara Garcia:** None. **P. Pacheco:** None.

Poster

223. Auditory Processing: Perception, Cognition, and Action

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Title: Rats and VRats: Using virtual rats to study the behavior and neural activity of freely moving rats in a complex environment

Authors: *A. KAZAKOV[1], M. M. JANKOWSKI[1], A. POLTEROVICH[1], J. NIEDIEK[1], I. NELKEN[1,2];
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Abstract: Reinforcement learning together with deep learning (RL-DL) is a powerful paradigm for solving real-world control problems. We developed two parallel setups to investigate

biological and computational reinforcement learning - The first one is a large interactive environment (RIFF, rat interactive fantasy facility; diameter, 1.6 m) where rats train to maximize rewards (food/water) over time. The RIFF operates according to a Markov Decision Process, based on the rat location and activity. The rats interact with the arena via 12 ports, comprised of a food or water dispenser, an air-puff valve and a nose poke detector ("interaction areas", IAs). The rat is tracked in real-time by a ceiling-fixed camera (30 Hz). We taught adult rats (Sabra, female, N=5) to behave as instructed by different sounds that are presented in the RIFF, in order to maximize rewards (food/flavored water) and to avoid air-puffs. The rats performed hundreds of short trials (10-30 seconds, each ending with a reward or a punishment) in a single session (1 hour long, over many weeks). Neural responses were recorded from insular or auditory cortices by 16 tungsten electrodes (flexDrive) with a Neurologger system (Deutron). The other setup is an in-silico replica of the RIFF, a computer model that performs similar tasks under similar environmental laws and constraints. The full observability of the virtual setup allows us to develop hypotheses that we test on the biological one. The RIFF was replicated in-silico as a 2D arena with similarly located IAs, driven by the same MDP. The virtual rat (VRat) is modeled as a point inside this arena, with constraints on acceleration and turns to resemble the biological rat. The VRat brain is comprised of an artificial neural network (Deep Q-Network, 300 neurons in total) that emits an action based on the observed state. The VRat was trained through reinforcement learning, and converged to near-optimal policies that were surprisingly similar to the policies of the biological rat as compared by reward return, trajectory features and action distribution. We currently investigate what causes the rats and VRats to converge to similar policies, and to what extent the internal representations (as judged by neural responses in the rats and by the full state of the neural network in the VRat) are related to each other.

Disclosures: **A. Kazakov:** None. **M.M. Jankowski:** None. **A. Polterovich:** None. **J. Niediek:** None. **I. Nelken:** None.

Poster

223. Auditory Processing: Perception, Cognition, and Action

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 223.22/K22

Topic: D.06. Auditory & Vestibular Systems

Support: ERC grant no. 340063 - RATLAND
Israel Science Foundation F.I.R.S.T. grant no. 1075/13

Title: Behavior of freely moving rats in a complex environment modeled by reinforcement learning with informational constraints

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Abstract: Behaviors of rats in natural environments vary in complexity and in their effectivity in reward collection. We used a large interactive environment ("RIFF", rat interactive fantasy facility; diameter, 1.6 m) in which freely moving adult female Sabra rats (N = 5) obtained food or water rewards from 12 ports. Rats had to time their motion and their nose-pokes according to different sounds in order to obtain rewards and to avoid air-puffs. At all times, rats could choose between several action sequences that differed in both complexity and reward sizes. The RIFF operates as a Markov Decision Process (MDP), where each of the rat actions shifts the environment from one well-defined state into another well-defined state. The theory of MDPs allows to find optimal policies which maximize the reward rate. In order to model behavioral complexity, we added to the MDP's value term a complexity term, where a learned policy's complexity is measured as the Kullback-Leibler divergence between the learned policy and a simple default policy. The model produced a family of policies that differ in their complexity, and realize the optimal reward rate at any given complexity. In the RIFF, all rats learned policies that ensured high reward rates, and different rats discovered different policies. Moreover, these policies shifted within 90-minute recording sessions. A model with information constraints qualitatively captured these different policies. Using likelihoods of recorded trajectories with respect to optimal policies of varying complexity, we estimated actual trade-offs between value and complexity. The shifting policies within sessions were reflected by a concurrent monotonic decrease of their complexities for the best-fitting optimal policies. Our results show that informational constraints are a promising approach for behavioral modeling, even in a scenario where the number of possible actions is high. It provides a large selection of optimal policies that capture much of the observed variability during a session and across animals in a uniform way. Since it is derived from first principles, it can readily be used in a wide range of applications.

Disclosures: J. Niediek: None. **M.M. Jankowski:** None. **A. Polterovich:** None. **A. Kazakov:** None. **I. Nelken:** None.

Poster

224. Human Auditory Processing II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 224.01/K23

Topic: D.06. Auditory & Vestibular Systems

Title: Classification of electroencephalographic oscillations during relative pitch imagery

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Abstract: Auditory imagery is associated with complex neural representations of direct and contextual information of an imaged tone. Though electroencephalography (EEG) has succeeded to read oscillatory representations of motor and visual imagery, fewer studies have focused on the auditory domain, especially in pitch imagery, presumably due to the difficulty of capturing direct information of imaged pitch such as tonotopy with EEG. Therefore, we examined classification of EEG oscillations for pitch imagery by focusing on relative pitch change from the baseline. During EEG recording, eleven healthy musically trained participants (three males and eight females, age = 19.5 ± 1.2) were first presented with a reference tone at a frequency of either 220 or 880 Hz. Subsequently, they were instructed to image a tone that was either one octave higher or lower than the reference tone based on a visual cue of either up or down arrow, respectively. The frequencies of imagery tones could thus be either 110, 440, or 880 Hz, where the 440-Hz imagery had two different contexts. Using debiased phase lag index (dwPLI) between combinational channels as feature values and linear support vector machine as a classifier, EEG data during imagery of two types of 440-Hz tones were classified with a rate of above 70% for all and 87.5% for the champion participant. Especially, dwPLI involving the parietal channels and at the alpha band showed significantly higher classification rates compared to dwPLI at the non-parietal channels and at other frequency bands, respectively. Since auditory imagery was reported to form a broad network involving the parietal area, we conclude that the network partially functions based on relative changes of imaged pitch using alpha band and that these neural representations of contextual information can be captured with EEG.

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Poster

224. Human Auditory Processing II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 224.02/K24

Topic: D.06. Auditory & Vestibular Systems

Support: JSPS Grant 16K 12456

Title: Relation between EEG rhythms and sensorimotor synchronization in auditory and visual modalities

Authors: *M. SASAKI, H. OKAWA, T. TANAKA;
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Abstract: Introduction: The neural mechanisms of beat processing have been studied for many years. It has been reported the functional role of induced beta oscillations during beat perception and suggested that the beta power rebound might reflect timing predictions of the next beat

(Fujioka et al., 2012). However, the role of beta rebound is still unclear because there is no behavioral evidence of predicted timing by participants, and the beta oscillations during beat perception have been studied only in the auditory modality. This study investigated the relation between beta oscillations and timing prediction both in auditory and visual modalities by using electroencephalogram (EEG). Methods: Twenty-one healthy young adults participated in the experiment. All participants had normal hearing and normal or corrected-to-normal vision. They gave their written informed consent, and the study was approved by the research ethics committee in the institution. In this experiment, participants were presented an auditory or visual beat stimulus and asked to perform a task of tapping to the beat and a task of concentrating on listening to or watching the stimulus with conscious of beat. The recorded EEG were bandpass-filtered (13-25 Hz) firstly. The short-time Fourier transform (STFT) was applied to identify the time course of power variation in the frequency domain. Moreover, tapping performance was evaluated by using tapping-asynchronies defined as the timing differences between onset timing of the beat and participant's tap. The correlation coefficient was calculated between pairs of tapping-asynchronies of every tap and midpoint of beta rebound obtained from individual-level beta power variations for each participant. Results: Significant beta desynchronization was observed after the beat onset of every stimulus. The correlation analysis results showed that significant weak correlations were found between tapping-asynchronies and midpoint of beta rebound at electrodes near the motor area for each stimulus. Conclusions: Our results support the hypothesis of beta oscillations that beta rebound reflects the timing prediction of the beat onset.

Disclosures: **M. Sasaki:** None. **H. Okawa:** None. **T. Tanaka:** None.

Poster

224. Human Auditory Processing II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 224.03/K25

Topic: D.06. Auditory & Vestibular Systems

Support: Guarantors of Brain
Association of British Neurologists

Title: The queen square tests of auditory cognition: Defining hearing deficits and disability in dementia

Authors: ***J. JOHNSON**, C. HARDY, H. SIVASATHIASEELAN, E. BENHAMOU, M.-C. REQUENA-KOMURO, L. RUSSELL, C. GREAVES, J. ROHRER, D.-E. BAMIOU, J. WARREN;
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Abstract: Hearing impairment has emerged as a potent association of cognitive decline in dementia and a promising treatment target. To realise this promise, we need to resolve fundamental questions concerning the roles of peripheral versus central auditory deficits in different dementias, which hearing measures best capture cognitive symptoms and daily life disability and whether hearing measures predict disease evolution. To begin to address these issues, we designed a novel psychoacoustic battery - the Queen Square Tests of Auditory Cognition (QSTAC) - that systematically assesses a range of peripheral and central hearing functions, including pure tone detection, otoacoustic emissions, speech-in-noise and gap-in-noise perception, spectrotemporal pattern analysis, spatial sound localisation, environmental sound recognition, auditory emotion processing and dichotic listening. Customised auditory symptom questionnaires (completed by patients' caregivers) capture daily-life hearing-related disability and care burden. The QSTAC has been administered to well-defined cohorts of patients representing canonical syndromes of Alzheimer's disease and progressive aphasia: diseases in which auditory symptoms are typically early and prominent. Patient performance is referenced to a cohort of healthy age-matched controls and adjusted for general disease factors. Syndromic signatures of peripheral and central auditory dysfunction were identified. Alzheimer's disease was associated with prominent impairments of auditory scene analysis. The progressive aphasias were principally associated with deficits of sound pattern analysis, sound identity and emotion recognition: patients with nonfluent/agrammatic primary progressive aphasia performed worse on pure-tone audiometry than healthy older individuals or patients with Alzheimer's disease and showed increased functional inter-aural audiometric asymmetry. In addition, patients with semantic variant progressive aphasia frequently exhibited striking abnormalities of sound reactivity, contributing to daily life disability. Taken together, our findings suggest that major dementias have characteristic and differentiated auditory phenotypes, reflecting a complex interplay of peripheral hearing and auditory cognitive dysfunction.

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Poster

224. Human Auditory Processing II

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Program #/Poster #: 224.04/K26

Topic: D.06. Auditory & Vestibular Systems

Support: Research Council of Norway, Grant 240389
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Title: Auditory prediction and prediction error in self-generated tones

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Abstract: Sensory consequences of actions are predicted by the brain to prepare cortical areas and reduce sensory processing activity (Knolle et al., 2013). This has been has been thought to rely on action-related forward prediction by an internal forward model (Wolpert, 1997). In the same vein, the predictive coding framework suggests that perception is based on internal models making predictions about sensory events, but based on statistical probabilities (Friston, 2010). If predictions are violated, a prediction error signal is transmitted to revise the generative model.
In the current study we investigated sensory predictions and prediction errors related to self-generated sounds. We used a self-paced, two-choice random generation task (Iwanaga and Nittono, 2010), infrequently inducing deviant outcomes of voluntary action. Participants repeatedly pressed a right and a left button normatively associated with a 70 ms long 1 kHz and 2 kHz tone, respectively. They were instructed that their button presses should be random, at a regular but self-paced tempo of one press per 1-2 s, and that they should press both buttons with equal probability. They were informed that the tones are task-irrelevant. Five blocks were run, consisting of 159 trials each. After 20 button presses, occasional deviants occurred, inverting the button-tone association, with a probability of 13,2%.
We used intracranial EEG (iEEG) data recorded from 10 adult patients with electrodes localized in frontal and temporal lobes. The patients had drug resistant epilepsy and were undergoing presurgical monitoring via implanted stereotactic electrodes. Electrode coordinates were obtained from coregistered MRI and CT images using iElectrodes toolbox (Blenkmann et al., 2017). Channels were bipolar referenced and the time-frequency representations were calculated by Morlet wavelets. Initial results indicate that prediction errors elicited high frequency band activity (HFA, 75-145 Hz) in a distributed network including the insula, temporal and prefrontal cortices. The prediction of a tone change was accompanied by enhanced alpha power, presumably inhibiting sensory processing of the expected tone (Mueller et al., 2014). The high spatial and temporal resolution of iEEG data allows to reveal neurophysiological mechanisms underlying predictions and prediction errors.

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Poster

224. Human Auditory Processing II

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Program #/Poster #: 224.05/K27

Topic: D.06. Auditory & Vestibular Systems

Support: ICMR- BMS/FW/CMB/2014-23710/oct-2014/14/DL/GOVT
Institutional research grant (All India Institute of Medical Sciences, New Delhi)

Title: Morphological development of human cochlear nucleus - A stereological study

Authors: *S. SAINI[1], T. G. JACOB[1], A. THAKAR[2], K. K. ROY[3], T. ROY[1];
[1]Anat., [2]Otorhinolaryngology, [3]Obstetrics and Gynaecology, All India Inst. of Med. Sci., New Delhi, India

Abstract: The cochlear nuclear (CN) complex occupies an important location in the hierarchy of functional components of the auditory pathway. The present study aims to investigate the pre and postnatal developmental changes in human CN to understand the important time points of maximal cell division, growth, differentiation and apoptosis. Twenty nine human pre and post natal brainstems were obtained from departments of Obstetrics & Gynaecology and Forensic Medicine with proper ethical permission. The samples were dissected, fixed, cryoprotected, serially sectioned and stained with cresyl violet (CV) and immunostained for the expression of NeuN (ab177487;1:500), GFAP (ab10062; 1:1500), Ki-67 (ab 8191;1;500). TUNEL staining was performed to see the apoptosis. These sections were used independently for estimating the total number of cells by the Optical Fractionator probe on StereoInvestigator software (Microbrightfield Inc. VT, US). The total volume of CN was estimated by Cavalieri principle. The data were divided into four groups (18-20 Weeks of gestation (WG) (group 1), 21-24 WG (group 2), 25-28 WG (group 3), and 29 WG onwards (group 4) and was analysed by Kruskal-Wallis followed by posthoc test for multiple comparisons. The volume increased significantly from 0.6 mm^3 (0.4 - 0.7) at 18WG to 4.4 mm^3 (0.4- 0.7) at 2-years after birth. There was a significant difference in the volume group 1vs 3 (p= 0.03) and 4 (p= 0.001) and group 2 vs 4 (p= 0.03). The median value with interquartile range for NeuN, GFAP, Ki-67, TUNEL for 18-20 WG were 30694(19166-32765), 27877(22946-39049), 33359 (10342-47239), 9562 (18091-33750) respectively and increased significantly to 83125 (66879-97533), 106193(81943-1233770), 79635 (54165- 98942), 61889 (50717 -78125) for group 4. A significant difference in NeuN was seen in the groups between 1 vs 4 (p=0.001) and 3 (p= 0.02) and group 2 vs 4 (p=0.00). For GFAP a significant difference was noted in group 1 vs 3 (p=0.04) and 4 (p=0.001), 2 vs 4 (p=0.006). For Ki-67 there was no individual difference between the groups except group 1 vs 4 (0.009) group 2 vs 4 (p=0.01) and group 3 vs 4 (p=0.02). For apoptotic cells (TUNEL) there was a significant difference between group 1 vs 3 (p=0.03) also between group 1vs 4

(p=0.005). The study provides baseline data and critical time points in the morphological development of the human CN that confides our earlier data on developing CN and cochlear nerve.

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Poster

224. Human Auditory Processing II

Location: Hall A

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Program #/Poster #: 224.06/K28

Topic: D.06. Auditory & Vestibular Systems

Support: U01 (NS098976)
a grant from the J.S McDonnell Foundation

Title: Phase of ongoing EEG oscillations predict auditory perception

Authors: *I. T. TAL[1], M. LESZCZYNSKI[2], N. MESGARANI[2], C. E. SCHROEDER[3];
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Abstract: Visual perception has been shown to fluctuate in a rhythmic manner, pointing towards an internal sampling frequency of the system. For example, visual detection threshold relies on the phase and amplitude of the ongoing EEG oscillations in the theta (4-7Hz) and alpha (8-12Hz) bands (e.g. Busch and VanRullen, 2009, 2010). However, it is unclear whether these oscillations are unique to vision or whether auditory performance also depends on the phase of ongoing oscillations. Recently, Ho et al. (2017) found that auditory perception oscillates at different frequencies (and phases) for the two ears. However, there is no evidence that ongoing EEG oscillations predict auditory perception. We hypothesized that the phase of pre-target ongoing oscillation will predict performance in an auditory discrimination task. Correct and incorrect will show different pre-target phase distributions. Participants (N=18) underwent EEG recording while performing an auditory pitch discrimination task between 2 target tones (2kHz and 2.5 kHz) each lasting 10 ms. Each trial started with white noise lasting 100 milliseconds to reset the phase of ongoing auditory oscillations. Then, a target tone was presented monaurally at a random time point between 1-2 seconds following the onset of the white noise. Phase distributions across trials were compared between correct and incorrect responses using inter-trial phase coherence (ITC). By calculating the bifurcation index, we found that before stimulus onset, each of the two distributions exhibited significant phase concentration, but at different phase angles. This effect was strongest in the theta and beta frequency bands. This finding indicates that auditory

perception fluctuates over time along with the phase of ongoing EEG activity in the absence of neural entrainment, showing that oscillations in performance are not specific to vision. The results support the notion that ongoing oscillations shape our perception, possibly by providing a temporal reference frame for neural codes.

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Poster

224. Human Auditory Processing II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 224.07/K29

Topic: D.06. Auditory & Vestibular Systems

Support: Marie Skłodowska-Curie grant agreement number 722046

Title: Enhanced anticipatory predictions in the auditory system of individuals with tinnitus

Authors: M. PARTYKA[1], G. DEMARCHI[1], W. SEDLAY[2], W. SCHLEE[3], ***N. WEISZ**[1]; [1]Ctr. for Cognitive Neurosci., Univ. of Salzburg, Salzburg, Austria; [2]Univ. of Newcastle, Newcastle, United Kingdom; [3]Univ. of Regensburg, Regensburg, Germany

Abstract: A prevailing view on tinnitus sees this condition to result from hyperexcitability / -synchrony in subcortical and cortical auditory processing regions resulting from deafferentation due to hearing damage. Despite its merits especially in animal models, there is no strong and consistent evidence base in humans to support this view. Furthermore, this mechanistic account cannot explain several open issues that plague tinnitus research, in particular: Why does only a subset of individuals with hearing loss develop tinnitus? Why is after noise trauma tinnitus transient in most individuals, but becomes chronic in a few? Developing answers to these fundamental questions will be a key in also developing innovative treatments and identifying individuals at risk to develop (chronic) tinnitus. Recently, predictive processing models of tinnitus have been put forward that suggest the default prediction of silence to shift to a prediction of sound. This is an appealing hypothesis, since it does not require any hyperexcitability / -synchrony in the long term and could explain the notorious therapy-resistance of tinnitus. Extending this notion we propose that individuals that develop tinnitus may generally rely more strongly on their internal (prediction) models as compared to the actual auditory evidence. This would be a predisposing (~trait-like) factor to shift their default prediction towards tinnitus following e.g. a hearing damage. To pursue this idea requires a powerful methodological approach to uncover prediction processes in the auditory system.Recently we developed an MEG paradigm, that combines a regularity modulation and omission paradigm. Using an MVPA approach, we show that (anticipatory) predictions in the

auditory system are tonotopically (i.e. carrier-frequency) specific. In the present study, we applied this powerful base paradigm to individuals with tinnitus. Our results show strong differences in anticipatory periods, with activity generated by "tinnitus brains" containing a stronger modulation of carrier-frequency specific information with increasing regularity of the sound sequence. Since no differences between groups were present with regards to the general decoding of carrier-frequency in random sound sequences, our effects point clearly to an altered processing of statistical regularities in tinnitus. Overall, these results support our notion of an increased reliance on internal models in auditory processing of individuals with tinnitus. However, more work will be needed to establish this process as a factor that predisposes tinnitus.

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Poster

224. Human Auditory Processing II

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Topic: D.06. Auditory & Vestibular Systems

Support: R01DC012947
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Title: Eye movement-related contextual modulation of auditory cortical activity

Authors: ***A. BARCZAK**[1], M. N. O'CONNELL[1], T. MCGINNIS[1], S. A. NEYMOTIN[1], C. E. SCHROEDER[1,2], P. LAKATOS[1,3];
[1]Nathan Kline Inst., Orangeburg, NY; [2]Columbia Univ. Col. of Physicians and Surgeons, New York, NY; [3]NYU Langone Med. Ctr., New York, NY

Abstract: The auditory and visual sensory systems are both used by the brain to obtain and organize information from our external environment, yet there are fundamental differences between these two systems. For example, while actively searching a scene, information is acquired using systematic patterns of fixations and saccades, which are controlled by internal motor commands. In this condition, sensory input occurs in volleys, strictly tied to the timing of eye movements. The auditory system in contrast, does not use such an overt motor sampling routine so the relationship between sensory input timing and motor activity is less clear. Previous studies of primary visual cortex (V1) in nonhuman primates have shown that there is a cyclical modulation of excitability tied to the eye movement cycle. Eye movements modulate visual processing in V1 such that stimulus responses are larger when stimuli are presented just after fixation onset as opposed to later during the fixation. The analysis of neuronal oscillations in V1

suggests that this eye movement-related modulation of excitability stems from phase reset in the theta-alpha frequency range. We hypothesized that if eye movements provide a supramodal temporal context for environmental information then we should also see eye movement-related modulation of oscillatory activity in primary auditory cortex (A1) as nonhuman primates shift their gaze around their surroundings. To examine this eye movement related activity in A1, we used linear-array multielectrodes to record cortical laminar neuroelectric activity profiles while subjects sat in a darkened silent chamber. Analysis of oscillatory activity in A1 suggests that, as in visual cortical regions, saccades are indeed capable of resetting neuronal oscillations in auditory cortex. When compared to the laminar-angular patterns of phase reset by auditory stimuli, a consistent difference was observed. Our results indicate that besides environmental multisensory inputs, motor sampling patterns like saccades can alter auditory cortical excitability despite the sampling of auditory inputs rarely being coherent with any motor action.

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Poster

224. Human Auditory Processing II

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Program #/Poster #: 224.09/K31

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant 3R01HD083310

Title: Alpha oscillatory activity reflects infants' emerging link between sounds and cognition

Authors: *K. WOODRUFF CARR[1], D. R. PERSZYK[1], E. S. NORTON[2], J. L. VOSS[3], S. R. WAXMAN[1];
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Abstract: Infants must identify which acoustic signals warrant attention and how these are linked to the world around them. By 3-4 months of age, infants have already initiated this link: listening to vocalizations of humans and nonhuman primates facilitates their ability to form object categories. But by 6 months, infants have tuned this broad initial link to include human speech alone; nonhuman vocalizations no longer support categorization. To identify the neurocognitive mechanisms that underlie this process, we collected EEG activity from healthy, full-term 4- and 6-month-olds (N=17 per group) during a passive listening task. We compared oscillatory activity elicited by a) infant-directed speech (IDS), a signal consistently linked to infant cognition, b) nonhuman primate (LEMUR) vocalizations, a signal linked to cognition at 4, but not 6, months, and c) backwards IDS (BW-IDS), a signal that is not linked to cognition at

any age. Epochs were extracted relative to stimulus onset and baseline corrected on a trial-by-trial basis to a pre-stimulus period. Activity in the alpha band is prominent from early infancy onwards, and has been linked to social and attentional engagement in infants. Given the inverse relation between alpha activity and cortical activation, we predicted that signals that support cognition will elicit decreased alpha power. Differences in event-related spectral perturbations in infants' centroparietal alpha (4-8Hz) power in response to these signals converges well with behavioral evidence. In 4-month-olds, alpha power decreased in response to IDS and LEMUR to a similar magnitude, relative to BW-IDS. In 6-month-olds, responses to IDS and LEMUR diverged: alpha power also decreased in response to IDS but increased in response to LEMUR; alpha power did not change in response to BW-IDS. These results suggest a neural correlate that underlies infants' earliest language-cognition links. The same signals elicited different neural responses in 4- and 6-month-olds. At 6 months, infants listening to IDS present neural signatures of engagement. In contrast, infants listening to LEMUR present neural signatures of disengagement. We discuss how speech-evoked neural oscillations might facilitate a learning state that supports infant cognition and how exposure guides infants to specify which signals, from an initially broader set, they continue to link to cognition.

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Poster

224. Human Auditory Processing II

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Topic: D.06. Auditory & Vestibular Systems

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the Pew Charitable Trusts Pew Biomedical Scholars Program

Title: Neural representation of speech in spatial multi-speaker speech perception

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Abstract: Humans can attend to speech of a talker in an acoustically complex, spatial separated multi-talker environment. How the human auditory cortex encodes speech of simultaneous spatially separated talkers and how attention to the location of a talker modulates the neural response is unknown. Here, we record intracranially from the auditory cortex of subjects engaged in a listening task with two simultaneous spatially separated talkers, and each spatial talker stand alone. We demonstrate that the location of speech played in quiet has little effect on

its encoding in the human auditory cortex, however, irrespective of attention, in the presence of spatial interfering talker the neuronal tuning narrows to the speech of contralateral talker for the majority of electrodes. Moreover, we show what aspects of the neural response are modulated by attention to the direction of speech and by attention to the talker. Our results demonstrate how the neuronal tuning changes in presence of spatially competing talkers and how the spatial auditory attention modulates the neuronal response.

Disclosures: P.M. Patel: None. **J.L. Herrero:** None. **A.D. Mehta:** None. **N. Mesgarani:** None.

Poster

224. Human Auditory Processing II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 224.11/K33

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant R01 DC016119

Title: Binding the components of a complex stream during segregation

Authors: *M. REZAEIZADEH[1], H. LU[3], A. J. OXENHAM[3], S. A. SHAMMA[2,1,4];
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Abstract: Humans and other animals can segregate a targeted sound from background interference and noise with remarkable ease. It is hypothesized that attention is essential for this process to occur in a listener's brain, and that the *temporal coherence* of the acoustic features of the target, and their incoherence from those of other sources, are the two key factors that induce the binding of the target features and its emergence as the foreground sound source. Specifically, the *temporal coherence* principle implies that acoustic features underlying the perceptual attributes of a sound emanating from a single source (e.g. its pitch, timbre, location, loudness) fluctuate coherently in power over time, and that the attentive listener tracks and utilizes this coherence to extract perceptually the source. This predicts that attentive listening induces (1) mutual excitatory influences among neurons responding to coherent features, hence causing them to mutually enhance their responses; and, by contrast, that (2) simultaneous mutual suppression would build up among incoherently responding neurons, causing them to diminish. In this study, we test these predictions in human subjects by measuring EEG responses while they segregate streams of complex sounds ranging from simple harmonic and inharmonic tone complexes to speech mixtures. To gain a view of the responses to individual frequency components in the mixtures, we exploited the modulatory-effects of ongoing and persistent attention on the

responses to a probe tone to measure the enhancement and suppression of the complex components while subjects were instructed to selectively attend to various target streams. Preliminary results are consistent with the predictions of *temporal coherence* and thus provide evidence for how the brain can online perceptually segregate complex mixtures, and hence enhance the ability of listeners to track target sounds despite severe noise and interference.

Disclosures: **M. Rezaeizadeh:** None. **H. Lu:** None. **A.J. Oxenham:** None. **S.A. Shamma:** None.

Poster

224. Human Auditory Processing II

Location: Hall A

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Program #/Poster #: 224.12/K34

Topic: D.06. Auditory & Vestibular Systems

Support: European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement number 743482
Medical Research Council UK, grant number SUAG/008/RG91365
British Academy/Leverhulme, grant number SRG18R1\180733

Title: Is there a contribution of true oscillatory activity in the processing of rhythmic speech sounds? Evidence from MEG and tACS

Authors: *B. ZOEFEL, S. VAN BREE, E. SOHOGLU, M. H. DAVIS;
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Abstract: A range of existing findings in the literature are consistent with oscillatory neural mechanisms contributing to speech perception. For instance, intelligible speech evokes more coherent brain responses at low frequencies (~4 Hz) than unintelligible speech. Furthermore, word report scores depend on the phase lag between transcranial alternating current stimulation (tACS) and the rhythm of heard speech.
Nonetheless, putative oscillatory effects on speech processing can also be explained as due to neural activity evoked by speech; neural responses will appear to be rhythmic since they are evoked by a rhythmic stimulus. To distinguish these views, we tested whether rhythmic sensory (Experiment 1) or electrical (Experiment 2) stimulation produces sustained oscillatory responses which continue after the end of the stimulus. Effects of previous rhythmic stimulation which continue in the absence of stimulation can provide stronger evidence for true involvement of oscillatory activity.
In Experiment 1 (N = 20), we measured brain responses with magnetoencephalography (MEG) during and after rhythmic intelligible and unintelligible speech sequences, presented at one of two different rates (2 Hz, 3 Hz). Although MEG responses, specific to the stimulation rate,

briefly outlasted the offset of the speech stimulus, they were too short to be distinguishable from those evoked by the first omission of an expected stimulus. Moreover, the sustained response did not depend on the intelligibility or the length of the preceding speech sequences, factors that we might expect to influence neural oscillations.
In Experiment 2 (N = 19), participants were asked to report spoken words embedded in noise. These words were presented at six different phase delays, during or immediately after bilateral tACS at 3 Hz over auditory regions. We found that word report accuracy fluctuates rhythmically after the offset of tACS, at a frequency corresponding to the stimulation. This sustained, phasic modulation of performance was numerically larger than, but not significantly different from, that observed for tACS that continued at the time of speech presentation. These findings show that modulation of neural oscillations produces a perceptual effect that is sustained beyond the end of tACS.

Disclosures: **B. Zoefel:** None. **S. van Bree:** None. **E. Sohoglu:** None. **M.H. Davis:** None.

Poster

224. Human Auditory Processing II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 224.13/K35

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant 5R01DC015260-03
NIH gRANT UL1-RR024979

Title: Multi and single unit activity in human Heschls gyrus during speech perception and production

Authors: *A. RAMIREZ-CARDENAS[1], R. BEHROOZMAND[2], C. K. KOVACH[1], P. E. GANDER[1], R. KELLEY[1], K. V. NOURSKI[1], H. OYA[1], H. KAWASAKI[1], M. A. HOWARD, III[1], J. D. GREENLEE[1];
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Abstract: Suppression of responses to self-generated sounds within auditory cortex has been extensively investigated in human neuroimaging and animal neurophysiology studies. Vocalization suppresses activity in auditory cortex relative to activation elicited by speech playback. This modulation of auditory neural responses is deemed essential for monitoring speech production. Auditory suppression is thought to be subserved by a circuit that compares forward predictions with actual sensory feedback. To study the neural networks involved in speech control, we delivered an online perturbation in the auditory feedback while subjects vocalized. Auditory feedback was shifted in either the frequency or time domain for a variable

short period in pseudorandomized trials. Voice was recorded and subsequently played back to the subjects for comparisons between auditory and motor activity. We recorded LFPs, multiunit and single-unit activity from Heschl's gyrus (HG) in 17 neurosurgical patients undergoing epilepsy surgery evaluation. We found that neural activity was differentially modulated by the start of auditory feedback in posteromedial and anterolateral HG. Moreover, multiple single neurons showed differential responses to auditory feedback during listening vs. speaking conditions. Consistent with animal studies, some of these neurons exhibited an attenuated response to auditory feedback during vocalization. This suppression, relative to baseline activity started at least 100 ms before the onset of auditory feedback and peaked shortly after. This finding is interpreted as a potential neuronal correlate of speaking-induced suppression of human auditory cortex. Moreover, we found multiple neurons in which excitatory neuronal responses were elicited during both listening and speaking conditions. We further analyzed the frequency tuning properties of these two neuronal subpopulations in the auditory cortex and investigated their role in error detection when auditory-feedback was perturbed. Based on previous reports from marmoset studies, we hypothesize that neurons exhibiting motor-induced suppression would play a prominent role in the detection of auditory-feedback perturbations. Moreover, we compared the behavioral contribution of these neuronal subpopulations in the trial-by-trial motor responses to perturbed auditory feedback. Finally, we analyzed the extent to which single and multiunit spike responses in the human HG could predict the LFP responses extracted from the same and adjacent recording sites. Our results represent the first report of spiking activity in human auditory cortex during speech perception and production.

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Poster

224. Human Auditory Processing II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 224.14/K36

Topic: D.06. Auditory & Vestibular Systems

Support: JSPS Grant 16K12456

Title: Effect on entrainment by selective attention to music and speech

Authors: *R. MATSUI, T. TANAKA;
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Abstract: Introduction: The cocktail party effect enables us to selectively focus our auditory attention to a particular speech or music. The brain mechanism of auditory selective attention has

been well investigated. Horton et al. reported that cortical entrainment to speech changes by selective attention (Horton et al. 2013). Their study showed that, when two different speeches are presented simultaneously, cortical entrainment to the attended speech is stronger than to that the unattended speech. O'Sullivan et al. reported that the attended speech can be decoded from electroencephalogram (EEG) to identify to which speech is attended (O'Sullivan et al., 2015). Maidhof et al. investigated event-related potentials (ERPs) related to the syntactic process of music and language when music and speech are presented simultaneously (Maidhof et al., 2011). They reported that the ERPs don't differ depending on which of them is attended to. However, it remains unclear whether the direction of selective attention influences cortical entrainment to speech and music. We investigate by EEG the cortical entrainment by selective attention to music and speech while music and speech were presented simultaneously.
Methods: Seventeen subjects participated in the experiment. All participants were healthy and had normal hearing. They were given an informed consent, and the study was approved by the research ethics committee in the institution. We prepared pieces of piano music and speeches of Japanese fairly tales, and recorded EEG while each participant was listening to music and speech simultaneously. The experiment consisted of 60 trials, each of about one minute in length. During the trial, they were instructed to attend to either the music or the speech of the presented stimulus. To model the selective attentions, support vector machine (SVM) was trained to classify whether the participant attended to speech or music by using features extracted from EEG with common spatial pattern method. To investigate entrainment, the cross-correlation function was calculated between filtered EEG (1-30 Hz) and envelope of the music or the speech.
Results: As a result of task classification by the SVM, ten participants achieved a classification accuracy of over 99%. The cross-correlation function averaged across trials, channels, and participants showed a negative peak at the time lag around 70 ms and a positive peak at the time lag around 140 ms. The peak to the speech when listening to speech was larger than that when listening to music, while the peak to music didn't differ depending on the attended stream.

Disclosures: **R. Matsui:** None. **T. Tanaka:** None.

Poster

224. Human Auditory Processing II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 224.15/K37

Topic: D.06. Auditory & Vestibular Systems

Support: NIDCD, DC014279

Title: Enhancing perception of speech in noise using electrical brain stimulation

Authors: *B. KHALIGHINEJAD[1], J. L. HERRERO[2], A. D. MEHTA[3], N. MESGARANI[4]; [1]Dept. Electrical Engineering, Columbia Univ., New York, NY; [2]Neurosurg., The Feinstein Inst. For Med. Res., Manhasset, NY; [3]Neurosurg., Hofstra North Shore LIJ Sch. of Med., Great Neck, NY; [4]Columbia Univ., New York, NY

Abstract: Accurate speech perception in noisy conditions requires a listener's auditory system to continuously monitor the incoming sound, tease apart the acoustic features of speech from the background noise, and selectively suppress the noise features relative to speech. This results in an internal representation of speech signal that enables robust speech comprehension unaffected by the changes in the acoustic background. However, it is not clear that what the causal role of different auditory regions is in generating such noise-invariant representation, and importantly, if it is possible to generate a better speech perception by electrical stimulation of specific regions.We stimulated different intracranial electrodes in auditory cortex of a neurosurgical patient using a bipolar, 3 mA, 50Hz signal. The simulation signal was four seconds on average and covered the entire speech in noise utterance. We systematically tested the intelligibility of speech during stimulation of electrodes in Planum temporale using a standard speech in noise intelligibility test (BKB-SIN) that consisted of 62 semantically balanced sentences. Half of the trials were chosen randomly for simulation and the other half were sham trials (no stimulation). The patient was asked to report the sentence and rate its quality using the mean opinion score (MOS) scale. The patient rated the speech quality significantly higher in stimulated trials compared to sham trials. In addition, speech intelligibility was significantly higher in stimulated compared to sham trials. Importantly, The patient reported reduced perceived background noise only when the electrodes in planum temporale were stimulated. The neighboring electrodes did not produce the same effect.While clinical constraints limit the applicability of this method to a small number of subject, the perceptual effects induced by brain stimulation provides valuable insights in the role of planum temporale and mechanisms of noise reduction process in human brain.

Disclosures: B. Khalighinejad: None. **J.L. Herrero:** None. **A.D. Mehta:** None. **N. Mesgarani:** None.

Poster

224. Human Auditory Processing II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 224.16/K38

Topic: D.06. Auditory & Vestibular Systems

Title: A comparison between electrical muscle stimulation and auditory stimulation in tempo-changing beat perception

Authors: *R. KONNO[1], P. SAVAGE[1], P. LOPES[2], S. FUJII[1];
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Abstract: Previous studies have reported that humans perceive and discriminate a pattern in time intervals (i.e., rhythm) more accurately with auditory stimuli than they do with visual stimuli [Repp & Penel, 2002; 2004; Grahn, 2012]. Recently, researchers have also been exploring how humans also discriminate timing on tactile and proprioceptive feedback. In particular, we leverage previous findings on vibrotactile feedback [Ammirante, et al., 2016] but turn to a new haptic modality: electrical muscle stimulation (EMS). EMS has been used for promoting the learning of motor movements [Lopes, et al., 2015; Ebisu, et al., 2017]. However it is unclear how human rhythm perception changes under EMS. In this study, we hypothesized that EMS promote human rhythm perception. We tested this hypothesis by investigating rhythm perception threshold under auditory stimulation, EMS, and combination of the two.
Ten individuals (four male, aged 19-23 years old) participated in this study. The Beat Interval Test in the Harvard Beat Assessment Test (H-BAT) [Fujii & Schlaug, 2013] was used to assess the perception threshold of beat interval changes. The test was presented under 1) auditory only, 2) EMS only, and 3) auditory+EMS conditions. A tempo-changing beat sound was provided to the participants under the auditory condition. Under the EMS condition, an electrical stimulation, which was synchronized with the sound onset timing, was provided to the flexor muscles of ring finger. Participants were asked to discriminate whether the tempo of inter-stimulus interval is getting faster or slower in the test. The order of conditions were counterbalanced among the participants.
We found out that the participants showed better discrimination of the tempo change in the auditory only condition compared to the EMS only condition ($p = 0.022$, using Wilcoxon Signed Rank Test). Participants also showed better discrimination in the auditory+EMS condition than the EMS only condition ($p = 0.005$). There was no significant difference between the auditory only and the auditory+EMS condition. Participants self-reported that the electro-mechanical delay (i.e., a lag between the muscle stimulation and the actual finger movement) made them hard to discriminate the tempo change. Our results did not support the hypothesis that EMS promote human rhythm perception. It may be difficult to find superiority of EMS compared to auditory stimulation in simple rhythm perception.

Disclosures: R. Konno: None. **P. Savage:** None. **P. Lopes:** None. **S. Fujii:** None.

Poster

225. Mechanisms of Retinal Circuit Assembly and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 225.01/K39

Topic: D.07. Vision

Support: NIH Grant EY022070

Title: A nitric oxide synthase expressing wide-field amacrine cell's role in shaping retinal output

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Abstract: Signals from amacrine cells (ACs), the inhibitory interneurons in the retina, are responsible for generating the distinct receptive field properties of the roughly 40 ganglion cell (GC) types. Despite the importance of ACs in defining circuit function, this large and diverse class of neurons remains poorly understood in part due to the difficulty in targeting cells for recording. In this study we target two populations of GABAergic ACs, identified in a transgenic mouse line as expressing neuronal nitric oxide synthase (nNOS). We investigate their receptive field properties and synaptic connectivity. Adult mice (P40-160) of either sex were used. We measured voltage and current responses from single ACs and GCs in dark-adapted flat-mount retina.
The two populations of wide-field ACs labeled by nNOS are distinguished by their morphology and receptive field properties. NOS1 are axon-bearing ACs stratifying in sublamina 5 (S5) of the inner plexiform layer (IPL) with sparse branches also in S1. Despite being bi-stratified, NOS1 are ON-type cells that fire bursts of action potentials during increases in contrast. NOS2 cells are mono-stratified within S3, and are ON-OFF type cells that display strong center inhibition. We optically stimulated ChR2-expressing NOS+ ACs to map their outputs. Light-flashes evoked GABAergic responses from several GC types, all of which had dendrites within S3 of the IPL, where NOS2 cells stratify. The most common postsynaptic partner was the W3 GC, known as a local motion detector. W3 GCs respond strongly to small objects moving across a static background (local stimulation) but are silent when the whole image moves (global stimulation), due to strong ON-OFF GABAergic input from the surround. Conversely, NOS2 cells are silent during local stimuli but are strongly activated during global stimuli. This receptive field structure combined with the direct inhibitory connection identified with the W3 GC leads us to propose that the NOS2 AC provides surround global inhibition to the W3 GC, aiding in its selectivity for small objects moving against a static background.

Disclosures: J. Leffler: None. **W. Taylor:** None.

Poster

225. Mechanisms of Retinal Circuit Assembly and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 225.02/K40

Topic: D.07. Vision

Support: NIH Grant EY015573
NIH Grant EY029869
Plum Foundation

Title: Mouse horizontal cells express subunits of rho-containing GABA(a) receptors

Authors: *A. A. HIRANO[1,2], S. A. BARNES[3,4,1], N. C. BRECHA[5,6,2];
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Abstract: Mammalian horizontal cells appear to use a novel hybrid GABA- and pH-mediated mechanism to modulate cone photoreceptor calcium currents (Grove et al., 2019). In this mechanism, a TPMPA-sensitive $GABA_A$ receptor (GABAR) produce a tonic GABA-activated current in the endings of rodent horizontal cells (Grove et al., 2019). Earlier characterization of rho-containing GABARs (previously called $GABA_C$ receptors) demonstrated immunoreactivity in the outer plexiform layer (OPL), but the identity of the cells expressing the rho subunits was not known. We set out to determine whether mouse horizontal cells express rho-containing GABARs using the highly sensitive RNAscope *in situ* hybridization (ISH) technique. In vertical sections of mouse retina, we observed that rho2 GABAR (Gabrr2) mRNA was co-expressed in the distal inner nuclear layer (INL) with calbindin (Calb1) mRNA, as well as that for synaptotagmin-2 (Syt2), two markers of horizontal cells. Combining immunohistochemistry for calbindin with RNAscope ISH with Gabrr1 and Gabrr2 probes confirmed expression of rho1 and rho2 subunits in the cell bodies of horizontal cells.
These findings suggest that GABARs consisting of rho1 and rho2 subunits are present in horizontal cells. This is consistent with earlier findings of rho2 immunoreactivity in the synaptic endings of mouse horizontal cells that invaginate rod and cone photoreceptor terminals, that are well positioned to participate in feedback inhibition to photoreceptors.

Disclosures: **A.A. Hirano:** None. **S.A. Barnes:** None. **N.C. Brecha:** None.

Poster

225. Mechanisms of Retinal Circuit Assembly and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 225.03/L1

Topic: D.07. Vision

Title: Adenylate cyclase 1 links calcium signaling to CFTR-dependent cytosolic chloride elevations in chick amacrine cells

Authors: *L. ZHONG, E. L. GLEASON;
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Abstract: The cystic fibrosis transmembrane conductance regulator (CFTR) regulates cytosolic Cl^- in amacrine cells (ACs). Our lab has demonstrated that nitric oxide (NO) elicits Cl^- release from acidic organelles by a CFTR-dependent mechanism (Krishnan, et al. 2017) which alters the synaptic response properties of postsynaptic ACs at GABAergic synapses (Hoffpauir et al. 2006). CFTR is an ATP- and cAMP/PKA-dependent Cl^- channel, however, the link between NO and activation of this channel in ACs is not known. Another effect of NO on ACs is to generate elevations in cytosolic Ca^{2+}. Hence, we test the hypothesis that a Ca^{2+}-dependent adenylate cyclase 1 (AdC1) serves as the functional link between NO and CFTR activation. ACs were cultured from 8 day embryonic chick (*Gallus gallus*) retinas. To monitor cytosolic Cl^- levels in ACs, the reversal potentials of GABA-gated currents ($E_{rev\text{-}GABA}$) were determined. ACs were held at -70 mV and voltage ramps (-90 mV to +50 mV, 200 msec) were delivered in the absence (for leak subtraction) and presence of GABA (20 µM). To confirm a role for Ca^{2+} in CFTR activation, ACs were recorded with 10 mM BAPTA, in the patch pipet and $E_{rev\text{-}GABA}$ was determined before and after NO. While in control cells (no BAPTA) the mean shift in $E_{rev\text{-}GABA}$ was 32.7 ± 3.4 mV, (n=6), with BAPTA, the shift in $E_{rev\text{-}GABA}$ was 1.7 ± 3.4 mV (n=5, $p<0.0001$, unpaired t-test) suggesting that a Ca^{2+} -dependent mechanism is involved. To establish a role for AdC, the effects of AdC inhibitors were explored. SQ 22536 (100 nM), a general AdC inhibitor, suppressed the shift in $E_{rev\text{-}GABA}$ (control shift 38.2 ± 3.6 mV; SQ 22536 shift 9.7 ± 3.6 mV; n=11 for both groups; $p<0.0001$, unpaired t-test). The AdC1 inhibitor ST 034307 was also effective; providing evidence for the involvement of AdC1, specifically (control shift 31.0 ± 4.3 mV; ST 034307 shift 13.1 ± 4.3 mV; n=9 each; $p<0.001$, unpaired t-test). Neither the AdC2 inhibitor SKF 83566 (10 µM) nor the AdC 3 and 5 inhibitor (NKY 80, 200 µM) inhibited the NO-dependent shift in $E_{rev\text{-}GABA}$ (control shift 41.5 ± 5.0 mV, n=11; SKF 83566 shift 42.1 ± 5.0 mV, n=10; p=0.9079; control shift 47.3 ± 5.3 mV, n=7; NKY 80 shift 38.7 ± 5.3, n=8; p=0.1254; unpaired t-tests). Two PKA inhibitors (H89 1µM, KT 5720 300 nM) inhibited the NO-dependent shift in $E_{rev\text{-}GABA}$ (control shift 30.2 ± 5.7 mV; H89 shift 12.3 ± 5.7 mV; n=9 for both groups; $p<0.01$; control shift 38.8 ± 4.3 mV, n=9; KT 5720 shift 9.8 ± 4.3 mV, n=8; $p<0.0001$, unpaired t-tests). Together, these results indicate a role for AdC1-dependent signaling in activation of CFTR and provide a link between NO-dependent cytosolic Ca^{2+} signaling and cytosolic Cl^- concentration.

Disclosures: L. Zhong: None. **E.L. Gleason:** None.

Poster

225. Mechanisms of Retinal Circuit Assembly and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 225.04/L2

Topic: D.07. Vision

Title: Cell types of the human retina and its organoids at single cell resolution: Developmental convergence, transcriptomic identity, and disease map

Authors: *C. S. COWAN[1,2], M. RENNER[1,2,3], B. G. SCHERF[1,2], D. GOLDBLUM[4], M. MUNZ[5], J. KROL[1], T. SZIKRA[1], P. PAPASAIKAS[2], R. CUTATT[3], A. WALDT[3], R. DIGGELMANN[6], C. PATINO-ALVAREZ[1], N. GERBER-HOLLBACH[4], S. SCHURIER[3], Y. HOU[1], A. SRDANOVIC[1], M. BALOGH[1], P. HASLER[4], A. KUSNYERIK[1], A. SZABO[7], M. STADLER[2], A. HIERLEMANN[6], H. SCHOLL[4], G. ROMA[3], F. NIGSCH[3], B. ROSKA[1,2,8];
[1]Inst. of Mol. and Clin. Ophthalmology Basel, Basel, Switzerland; [2]Friedrich Miescher Inst., Basel, Switzerland; [3]Novartis Inst. for Biomed. Res., Basel, Switzerland; [4]Ophthalmology, Univ. Hosp. Basel, Basel, Switzerland; [5]Friedrich Miescher Inst. For Biomed. Resear, Basel, Switzerland; [6]BSSE, ETH Zurich, Basel, Switzerland; [7]Dept. Anatomy, Histology, Embryology, Semmelweis Univ., Budapest, Hungary; [8]Univ. of Basel, Basel, Switzerland

Abstract: Human organoids are 3D cellular ensembles that are grown in vitro from adult or pluripotent stem cells and that reproduce some morphological, functional and transcriptomic features of human organs. Organoids engineered to harbor disease-causing mutations or grown directly from patient cells could provide mechanistic insights into diseases. However, organs consist of many specialized cell types and it is not well understood how closely human organoids recapitulate cell-type diversity and cell-type maturation of their target organs. The retina is a relevant model system to address these questions since its cell types have been extensively studied and retinal organoids can be grown from human pluripotent stem cells. Furthermore, retinal genes have been described that cause or contribute to many vision-impairing monogenic and complex retinal diseases, such as retinitis pigmentosa and macular degeneration.
Here, we report the development of retinal organoids from human induced pluripotent stem cells in large quantities. These organoids have three nuclear and two synaptic layers, a retinal pigment epithelium, and long outer segments. We obtained single-cell transcriptomes from 62,136 cells dissociated from developing human multilayered organoids at six different time-points spanning the 38 weeks of human gestation in vivo. Deep learning analysis of these transcriptomes revealed progressive maturation of retinal cell classes and showed that transcriptomes reached a stable, developed state by week 30. The rate of transcriptomic changes during organoid development was similar to the developing human retina in vivo.
We developed a procedure to obtain human retinas that experienced minimal hypoxia and maintained light responses and functional retinal circuits for hours ex vivo. We sequenced RNA from 116,089 single cells from the peripheral and foveal retina. Comparing periphery to fovea, we identified regional characteristics of cell types and, by comparing organoid to organ, we showed that transcriptomes of organoid cell types converge to those of adult human retinal cell types. In the context of cell types, we also compared developed organoids and adult human retinas in the expression of genes associated with retinal diseases. The resulting genetic disease maps showed retinal diseases to be cell-type specific and that cell-type specificity is preserved in organoids. These resources allow the identification of cellular targets for studying disease mechanisms in organoids and targeted repair in adult human retinas.

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Poster

225. Mechanisms of Retinal Circuit Assembly and Function

Location: Hall A

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Program #/Poster #: 225.05/L3

Topic: D.07. Vision

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Title: Mixed GABA glycine synapses mediate postsynaptic inhibition on mouse on alpha retinal ganglion cells

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Abstract: Output signals of neural circuits, including the retina, are shaped by a combination of excitatory and inhibitory signals. Inhibitory signals can act presynaptically to regulate transmitter release, or postsynaptically to truncate excitatory input. In the mammalian retina, inhibitory interneurons called amacrine cells contact bipolar cell terminals (presynaptic inhibition) and/or ganglion cell (GC) dendrites (postsynaptic inhibition) to shape retinal output. Amacrine cells typically utilize either GABA or Glycine to exert synaptic inhibition on target neurons. In this study, we determined the composition of postsynaptic inhibitory synapses on the soma and dendrites of mouse 'ON alpha' GCs - one of the well-characterized mouse retinal output neurons. ON alpha GCs were labeled in their entirety either by biolistic transfections or by use of the *Thy1*-YFP transgenic line, which labels a subpopulation of ON alpha GCs. By co-labeling ON alpha GCs with α3-containing GABAA receptors and α1-containing Glycine receptors (GlyRs), we found both these receptor clusters to be enriched within ON alpha GCs. Strikingly,

we found prominent co-localization between GABAAα3 and GlyRα1 receptor puncta across the soma and dendrites of ON alpha GCs. By assaying receptor expression at different developmental time-points we determined that expression of GABAAα3 receptors precedes GlyRα1 receptor expression on ON alpha GCs. Analysis of ON alpha GlyRα1 clustering in GABAAα3 knockout retinas revealed that GABAAα3 is necessary for recruitment of GlyRα1 receptors at inhibitory synaptic sites on the ON alpha GC. To study the ultrastructural arrangement of these putative 'mixed' inhibitory synapses across ON alpha GCs we performed serial block face scanning electron microscopy and mapped presynaptic amacrine terminals contacting ON alpha GCs. We found that ~40% of amacrine terminals providing input on ON alpha GCs had multiple transmitter release sites with the most common being dual release sites. 3D EM reconstructions of amacrine processes with 'dual' release pools revealed the identity of the wide-field amacrine interneuron driving this type of postsynaptic inhibition on mouse ON alpha GCs.

Disclosures: **A.A. Sawant:** None. **A. Bleckert:** None. **C. Gamlin:** None. **B.N. Ebbinghaus:** None. **W. Yu:** None. **R. Wong:** None. **R. Sinha:** None. **M. Hoon:** None.

Poster

225. Mechanisms of Retinal Circuit Assembly and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 225.06/L4

Topic: D.07. Vision

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Title: A characterization of genetic tools for the identification of intrinsically photosensitive retinal ganglion cells

Authors: *K. C. MIGUEL, R. H. IBRAHIM, E. CONTRERAS, A. M. MENZIE, T. BOZZA, T. M. SCHMIDT;
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Abstract: Intrinsically photosensitive retinal ganglion cells (ipRGCs) are a recently discovered, atypical type of photoreceptor in the mammalian retina. Unlike other retinal ganglion cells, ipRGCs express the photopigment melanopsin (Opn4), which allows them to respond to light even in the absence of rod and cone input. The six subtypes of ipRGCs (M1-M6) express different amounts of melanopsin, with some expressing amounts too low to visualize through immunohistochemistry or with a transgenic line where GFP is expressed under the melanopsin promoter ($Opn4^{GFP}$). To circumvent this limitation, researchers often take advantage of the

efficiency of the Cre-LoxP system to label greater numbers of ipRGCs. However, commonly used reporter lines label vastly different numbers of cells. We therefore sought to quantify and characterize the cell types labeled in two of the most commonly used reporter lines: $Opn4^{Cre}$; Z/EG (where ~3000 cells are labeled) and $Opn4^{Cre}$;$ROSA^{tdTomato}$ (where ~9000 cells are labeled). To do this, we use immunohistochemistry to characterize the number and types of retinal cells labeled in $Opn4^{cre}$; $ROSA^{tdTomato}$ mice. Our data show that all M1-M6 ipRGCs as well as other, distinct retinal cell types are labeled in the $Opn4^{Cre}$; $ROSA^{tdTomato}$ line. Using injections of a viral Cre-dependent reporter (mCherry), we show that the additional cells labeled in the $ROSA^{tdTomato}$ line likely express melanopsin in adulthood. Additionally, we use all three of these Cre-dependent reporters (Z/EG, $ROSA^{tdTomato}$, and viral mCherry) to characterize the melanopsin expression in a new Cre line ($Opn4^{EC\text{-}Cre}$) in which Cre expression is still driven by the melanopsin promoter but more of the melanopsin locus is left intact. Together, these results indicate that the $Opn4^{Cre}$ line is poised to manipulate a much larger population of retinal cells than is currently appreciated and suggests that there are several as of yet unidentified intrinsically photosensitive cell types in the mouse retina.

Disclosures: **K.C. Miguel:** None. **R.H. Ibrahim:** None. **E. Contreras:** None. **A.M. Menzie:** None. **T. Bozza:** None. **T.M. Schmidt:** None.

Poster

225. Mechanisms of Retinal Circuit Assembly and Function

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Program #/Poster #: 225.07/L5

Topic: D.07. Vision

Support: NIH intramural

Title: Classification of FoxP2-expressing retinal ganglion cells in mice

Authors: *A. FULLER, T. C. BADEA;
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Abstract: Retinal ganglion cells (RGCs) facilitate the transduction of visual information by integrating sensory input from the retina. Approximately 20 to 40 different RGC types have been recognized, but the exact number of types and the nature of their molecular determinants remain unclear. Earlier work has identified the Brn3/Pou4f family as a selective regulator of numerous RGC types. We previously recognized FoxP2 as a downstream regulator of Brn3a through RNA deep sequencing (Saigo, 2017 & Muzyka, 2018). Rousso et al (2016) recently described two pairs of RGC types marked by FoxP2 with subtypes marked by Brn3b and Brn3c; however, the exact transcriptional interactions of FoxP2 and Brn3a over the development and differentiation of RGCs remain unclear. Here, we survey various RGC types expressing FoxP2 in six flat-

mounted adult mouse retinas. We utilize a Cre-dependent GFP expression scheme resulting from an intravitreal infection of adeno-associated virus (AAV) 1 and 2 in the left and right retinas, respectively. We use morphometrics to characterize cell types, measuring arbor area, dendrite stratification, cell thickness, and cell eccentricity. Using this strategy, we can largely confirm the presence of four F-RGC types as characterized by Rousso et al (2016). However, we find more nuanced subtype distinction within these types, as well as a subgroup of alpha cells. We additionally find a notable assortment of non-RGC cell types, including amacrine cells, horizontal cells, astrocytes, and large numbers of Müller glial cells. Our findings indicate a wider array of FoxP2-expressing cell types than originally appreciated. We aim to continue this analysis by incorporating Brn3a into our examination of FoxP2-RGC type specification and exploring their transcriptional interactions.

Disclosures: A. Fuller: None. **T.C. Badea:** None.

Poster

225. Mechanisms of Retinal Circuit Assembly and Function

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Topic: D.07. Vision

Support: NIH Grant F31EY029593
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Title: An on-primary retinal ganglion cell receives off input via a heterotypic RGC gap junction

Authors: *S. COOLER[1], G. W. SCHWARTZ[2];
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Abstract: Gap junctions are found throughout the retina, and are known to exist between retinal ganglion cells (RGCs) of the same type. We have identified for the first time direct evidence of a gap junction network between RGCs of two different types: F-miniON and F-miniOFF. Each of these types receive excitatory synaptic input of only ON or OFF polarity, but both types exhibit both ON and OFF spiking for certain visual stimuli. We propose that these opposite-polarity signals are transmitted through gap junctions between the two types. This novel connectivity motif, with the asymmetric morphologies of these RGC types, creates asymmetric ON/OFF spatial receptive fields in individual RGCs. We are investigating RGC responses to various stimuli to determine the effects of this gap junction connectivity on visual feature selectivity.

Disclosures: S. Cooler: None. **G.W. Schwartz:** None.

Poster

225. Mechanisms of Retinal Circuit Assembly and Function

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Topic: D.07. Vision

Support: RO1EY019498
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NSF GRFP

Title: Synaptic basis of velocity tuning in direction selective circuits

Authors: *M. T. SUMMERS, M. FELLER;
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Abstract: Neural circuits in the sensory periphery must extract ethologically relevant features of the external world using hardwired, predominantly feedforward computations. However, these same circuits must flexibly accommodate a broad range of input conditions to reliably encode features of interest. To understand how circuits in the retina compute the direction of image motion, we compare the velocity dependence of direction selective computations performed by On and On-Off type direction selective ganglion cells (DSGCs). Here we use Hoxd10-GFP and Trhr-GFP mouse lines to perform 2-photon targeted cell-attached and whole-cell voltage clamp recordings from On and On-Off DSGCs in response to visual stimuli with varied spatiotemporal properties. We find that On-Off DSGCs remain directionally selective across velocities and stimuli, while On DSGC tuning is dependent upon stimulus type. We use whole-cell voltage clamp to investigate the synaptic mechanisms of direction selectivity, and find that On DSGCs receive slow, tonic excitation that is symmetric for preferred and null direction drifting bars whereas On-Off DSGCs receive fast, phasic, asymmetric excitation. Further, we find that both DSGC types receive transient, asymmetric inhibitory inputs of similar magnitude, but On DSGC inhibition becomes increasingly symmetric at high velocities. Finally, we use simple conductance modelling to probe the relative roles of asymmetric excitation and inhibition in computing direction selectivity at different speeds of image motion.

Disclosures: M.T. Summers: None. **M. Feller:** None.

Poster

225. Mechanisms of Retinal Circuit Assembly and Function

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Topic: D.07. Vision

Support: NIH NINDS F31 NS106756
NIH NEI R01EY019498-03

Title: Visual experience influences the orientation of DSGC dendrites while maintaining asymmetric wiring of the DS circuit

Authors: *M. Y. EL-QUESSNY[1], M. B. FELLER[1,2];
[1]Helen Wills Neurosci. Inst., [2]Mol. and Cell Biol., Univ. of California, Berkeley, Berkeley, CA

Abstract: Direction selectivity (DS) is a classical neuronal computation that occurs in many sensory systems. The circuit basis of this computation is best described in the retina, where DS is mediated by asymmetric wiring of a directionally selective ganglion cell (DSGC) with inhibitory interneurons called starburst amacrine cells (SAC). DS emerges before eye-opening, though components of the circuit may undergo refinement following visual experience. Here, we explore the impact of dark-rearing on the morphology and tuning of a population of ON-OFF ventral preferring DSGCs (vDSGCs) that have ventrally-oriented asymmetric dendrites in adulthood. First, around eye-opening at postnatal day 13-14 (P13-14), we found a significant misalignment of ON and OFF vDSGC dendrites from the ventral axis. In mice that were dark reared from birth to adulthood (P30), vDSGCs remained misaligned from the ventral axis. Despite this dramatic dendritic misalignment at eye opening, and in dark reared adults, vDSGCs maintain ventral directional preference. DS tuning in dark reared adults is correlated with asymmetric inhibition and spatially offset excitatory and inhibitory receptive fields. Hence, asymmetric wiring of SACs onto DSGCs is independent of postsynaptic dendritic morphology.

Disclosures: M.Y. El-Quessny: None. **M.B. Feller:** None.

Poster

225. Mechanisms of Retinal Circuit Assembly and Function

Location: Hall A

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Program #/Poster #: 225.11/L9

Topic: D.07. Vision

Title: Mechanisms underlying J-RGC's direction selectivity

Authors: *B. WANG, Y. ZHANG;
Inst. of Neuroscience, Chinese Acad. of Sci., Shanghai, China

Abstract: Sensory information processing is one of the most important functions in the nervous system. Retina is the first stage of visual processing. There are more than 30 subtypes of retinal ganglion cells (RGCs) in the mouse retina. Each subtype of RGCs integrates inputs from diverse types of presynaptic bipolar cells and amacrine cells to acquire different light response properties. Among all the aspects in visual processing, motion is a prominent visual event and direction selectivity is one of the best studied features. Investigations about On-Off and On directionally selective ganglion cells (DSGCs) suggest that the asymmetric inhibition from starburst amacrine cells is required in the emergence of direction selectivity. But until now little is known about how direction selectivity of J-RGCs, the Off DSGCs in the mouse retina, is generated. In this study we aim to identify the neural circuit and synaptic mechanism contributing to direction selectivity of J-RGCs. By whole-cell patch-clamp recording, we mapped the receptive fields of the excitatory and inhibitory inputs to J-RGCs. There are spatiotemporal slopes in the excitatory receptive field, almost identical to the slopes in the spiking receptive field of J-RGCs. Whereas the inhibitory receptive field is spatiotemporally symmetric and does not contain such slopes. Previous work has shown that the direction selectivity of J-RGCs can be explained by spatiotemporal slopes in the receptive field, thus excitatory inputs appear to be important in the generation of direction selectivity. We then tested the synaptic inputs during motion stimuli in the preferred and null directions. Stronger excitatory postsynaptic currents were observed during motion in the preferred direction, as can be predicted by the excitatory receptive field. However, we also observed stronger inhibitory postsynaptic currents during motion in the null direction, which cannot be explained by the inhibitory receptive field. Furthermore, when we blocked inhibition in the retina, J-RGCs' direction selectivity decreased significantly. We therefore conclude that both excitatory and inhibitory inputs during motion appear to be direction selective, and they both contribute to the direction selectivity of J-RGCs. Further study is needed to identify the origin of direction selectivity in the synaptic inputs, and to clarify the roles the excitatory and inhibitory inputs play. The results may provide an alternative strategy for direction selectivity in general.

Disclosures: B. Wang: None. **Y. Zhang:** None.

Poster

225. Mechanisms of Retinal Circuit Assembly and Function

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Topic: D.07. Vision

Support: R01EY026286
R01EY029121

Title: Single cell proteomic mapping of mice retinal cells by CyTOF (cytometry by time of flight)

Authors: *J. GAO[1], A. V. DEUSEN[2], C. DEPPMANN[1], E. ZUNDER[2], X. LIU[2];
[1]Biol., [2]Univ. of Virginia, Charlottesville, VA

Abstract: The goal is to establish a novel high-throughput technology to study different populations of retinal neurons in normal development and diseased conditions. The retina is the light sensitive tissue that lines the back of the eye, including photoreceptors, bipolar cells, horizontal cells, amacrine cells, retinal ganglion cells (RGCs) and glial cells. Each cell type contains many different subtypes, which exhibit distinct molecular features. Retinal cell classification by molecular profiles using conventional methods such as immunohistochemistry has been limited by the number of markers that we can use and much remains to be characterized. Here, we took advantage of a high-throughput intersectional technique - Mass Cytometry by Time of Flight (CyTOF) - to quantify more than 40 markers in millions of single cells in a few hours. CyTOF uses antibodies conjugated isotopically pure rare earth metals to characterize single cells that bound to the isotopes by a time-of-flight mass spectrometer. In this study, we have developed a dissociation protocol that gave us consistent and high yields of 4 million single cells/retina from adult wild type C57BL/6J mice. We further confirmed that the dissociated cells are consistently sampling the original tissue. We have also developed an antibody panel of 40 subtype-specific markers for mice retina and verified the specificity of each antibody by immunofluorescence, flow titration and CyTOF titration. Mass cytometry was performed, and the data was visualized using t-Distributed Stochastic Neighbor Embedding (t-SNE) and Uniform Manifold Approximation and Projection (UMAP). We are able to separate out major retinal cell populations and observe expected hierarchical benchmarks. We focused on identifying known and unknown RGC subtypes such as ipRGCs (intrinsically RGCs), four types of ooDSGCs (on-off directionally selective RGCs), and different subpopulations of alpha RGCs. In conclusion, we validated CyTOF as a powerful tool that allows comprehensive molecular classification of major retinal cell types and subtypes, which builds the foundation to examine population changes in normal and diseased conditions.

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Poster

225. Mechanisms of Retinal Circuit Assembly and Function

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Title: Developing tools to study the molecular landscape of intrinsically photosensitive retinal ganglion cell subtypes

Authors: *J. L. JAVIER[1], E. CONTRERAS[1], M. THOMSEN[2], A. M. MENZIE[1], T. BOZZA[1], T. M. SCHMIDT[1];
[1]Neurobio., Northwestern Univ., Evanston, IL; [2]Neurosci., Johns Hopkins Univ., Baltimore, MD

Abstract: Since their discovery, melanopsin-expressing, intrinsically photosensitive retinal ganglion cells (ipRGCs) have become recognized as bonafide photoreceptors. ipRGCs are a diverse class of cells consisting of six described subtypes, M1-M6. The six ipRGC subtypes have diverse morphology, electrophysiological properties, and brain projection patterns. ipRGCs are involved in both image forming (pattern detection, primarily driven by non-M1 ipRGCs) and non-image forming (circadian rhythm, pupillary light reflex, primarily driven by M1 ipRGCs) vision. These diverse properties and functions suggest underlying molecular diversity among the ipRGC subtypes, but molecular differences between subtypes are not well understood. Defining the molecular differences between ipRGC subtypes will allow for better understanding of the contribution of each subtype to visual processing and allow us to develop intersectional genetic tools to manipulate single RGC populations. To do this, our first aim was to establish single molecule fluorescent in situ hybridization (smFISH) as a method for validation of candidate molecular markers for ipRGC subtypes. Our second aim was to validate a new $Opn4^{FlpO}$ line for use in intersectional approaches to manipulate single ipRGC subtypes. The resulting data indicate that we can reliably perform smFISH and visualize RNA molecules both in genetically engineered mice with endogenous fluorescence and in combination with immunofluorescence in wild type mouse retinas, and that RGCs are labeled in the $Opn4^{FlpO}$ mouse line.

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Poster

225. Mechanisms of Retinal Circuit Assembly and Function

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Topic: D.07. Vision

Support: R01 EY 029323
R01 EY 017836-11
UMD Institutional Startup Funding

Title: Mapping synaptic fields of inhibitory interneurons with super-resolution imaging

Authors: *J. MINEHART, J. SINGER, C. SPEER;
Biol., Univ. of Maryland, College Park, MD

Abstract: A model system for exploring inhibitory interneuron microcircuitry is the mammalian retina. The outputs of ~50 subtypes of anatomically and functionally diverse inhibitory amacrine cells (ACs) shape the receptive field properties of ~30 downstream retinal ganglion cell (RGC) types, which provide visual input to subcortical targets in the brain. Mapping the synaptic connections of ACs, including the identities, sizes, and locations of individual input and output synapses, is essential for understanding the AC population, which is composed of narrow-field glycinergic and widefield GABAergic cells; these are thought to contribute to local or broader spatial scale computations, respectively. Here, we imaged and reconstructed the synaptic connections of genetically-targeted narrow-field and widefield ACs in the mouse retina using STochastic Optical Reconstruction Microscopy (STORM), a single molecule localization-based super-resolution imaging approach. By examining a combination of presynaptic, postsynaptic, and cell-type-specific immunomarkers, we quantified the location, density, and subsynaptic organization of synaptic inputs and outputs within genetically targeted AC microcircuits to generate molecule-specific synaptic maps of connectivity in the inner plexiform layer. Our results provide molecular connectomic maps of local and wide-field inhibitory cells within retinal circuits and further our understanding of how glycinergic and GABAergic microcircuit inhibition across multiple spatial scales shapes the receptive field properties of retinal outputs to the brain.

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Poster

225. Mechanisms of Retinal Circuit Assembly and Function

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Title: Argonaute 2 translocates to nucleus during the retinal development, and it is involved in the differentiation of specific neuronal subtypes

Authors: *M. I. MÓVIO[1], L. T. WALTER[2], A. H. KIHARA[1];
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Abstract: MicroRNAs (miRNAs) are small non-coding RNAs that control protein levels in post-transcriptional manner. The action of miRNAs depends on a well-orchestrated machinery that includes several elements. In this regard, it is particularly interesting the role of Argonaute-2 (AGO2), an essential protein in RNA-induced silencing complex (RISC). The main aim of this work was to characterize the role of AGO2 in retinal development. Both sexes Long Evans rats in early post-natal (P0) and adult (P60) ages was provided by UFABC vivarium, kept in 12:12h dark-light cycle. Animals were euthanized using intraperitoneal urethane (25%) and decapitation. Retinas were harvested to perform following experiments: i) real-time PCR (n=6) for gene expression, ii) western blotting (WB, n=5) for protein levels and iii) immunofluorescence (IF) (n=6) for protein levels and distribution. We also employed Manders' and Spearman coefficient analyses (n=6) for AGO2-nucleous colocalization. To induce AGO2 knockdown, morpholino oligo (MO) or scramble control oligo (Ctl) were injected in P0 subretinal space under anesthesia, and retinas were harvested after 7 days for WB (n=8), IF (n=6), and hematoxilin-eosin staining (HE, n=6). All procedures were approved by UFABC Animal Care Ethics Committee (16/2014) and results were measured using descriptive statistics and compared by paired t-test. PCR and WB revealed that both gene protein levels of AGO2 are lower at P0 (PCR: 2^{-1}=0.5-fold expression; $P<0.05$; WB: P0: 0.57±0.07 vs P60: 1.18±0.09 normalized optical density, $P<0.01$). IF and fractionated protein quantification showed no changes of AGO2 in cytosol, while in P60 AGO2 cumulates in nucleus (P0: 15.63±1.77 vs P60: 23.95±2.32, P<0.05*)*. When AGO2+ cells were analyzed, results showed that AGO2 localization depends on cell differentiation state. In P0, Spearman analysis showed lower nuclear AGO2 in immature cells than in mature (-0.04±0.22 vs 0.25±0.18, $p<0.05$), while Manders' revealed no changes in nuclear AGO2 in differentiated ganglion cells. MO treatment caused reduction of 52% in AGO2 protein levels, which induced several changes in the retina, as reduction in the thickness of the inner nuclear layer (Ctl:17.37±1.25 vs MO:13.69±1.38, P<0.05), increase in PKCα+ bipolar cells (Ctl: 14.83 ± 1.39 vs MO: 18.58 ± 0.67, P<0.01), CR+ amacrine cells (Ctl: 4.58 ± 1.56 vs MO: 11.17 ± 2.19, P<0.05), and decrease in ChAT+ amacrine cells (Ctl: 5.50 ± 0.58 vs MO: 6.00 ± 0.71, P<0.05). Taking together, our results revealed that AGO2 translocate to nucleus during retinal development, and its presence is essential for coordinated formation of retinal cell layers and differentiation of retinal subtypes.

Disclosures: M.I. Móvio: None. **L.T. Walter:** None. **A.H. Kihara:** None.

Poster

225. Mechanisms of Retinal Circuit Assembly and Function

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Title: GABA transporters and metabotropic receptors interact to regulate synaptic transmission in a direction-selective retinal circuit

Authors: *J. POTTACKAL[1], J. H. SINGER[5], J. B. DEMB[1,2,3,4];
[1]Interdepartmental Neurosci. Program, [2]Dept. of Ophthalmology and Visual Sci., [3]Dept. of Cell. and Mol. Physiol., [4]Dept. of Neurosci., Yale Univ., New Haven, CT; [5]Dept. of Biol., Univ. of Maryland, College Park, MD

Abstract: Synaptic GABA release subserves essential operations in neural circuit computations. For example, in the mouse retina, GABA release from starburst amacrine cells (SACs) suppresses responses to null-direction motion in postsynaptic direction-selective ganglion cells (DS GCs). Whereas the mechanisms and functions of GABA release in this circuit have been well studied, those of GABA clearance, mediated by plasmalemmal GABA transporters, remain poorly understood. Here, we combined electrophysiology, optogenetics, and pharmacology to elucidate the role of GABA transporter 3 (GAT-3) in synaptic transmission in a retinal DS circuit. During channelrhodopsin-2 (ChR2)-mediated stimulation of SACs, pharmacological blockade of GAT-3 unexpectedly reduced the amplitude of GABAergic inhibitory postsynaptic currents (IPSCs) recorded in ON-OFF DS (ooDS) GCs. This reduction in IPSC amplitude was blocked by co-application of the $GABA_B$ receptor antagonist CGP 52432 and reproduced by sole application of the $GABA_B$ receptor agonist baclofen, suggesting that GABA clearance by GAT-3 limits activation of presynaptic $GABA_B$ receptors that suppress GABAergic transmission. During conventional visual stimulation, GAT-3 blockade similarly reduced IPSCs in ooDS GCs during null-direction motion; however, under these conditions, spiking responses in ooDS GCs were unexpectedly reduced at low contrasts. Remarkably, we observed a concurrent reduction in excitatory PSCs (EPSCs). In concordance with this result, GAT-3 blockade dramatically reduced cholinergic EPSCs in ooDS GCs during ChR2-mediated stimulation of SACs; as with ChR2-evoked IPSCs, the effect on EPSCs was blocked by co-application of CGP 52432 and reproduced by sole application of baclofen, implicating $GABA_B$ receptors in heterosynaptic suppression of cholinergic transmission. Together, these results identify GAT-3 as a critical regulator of both inhibitory and excitatory synaptic function in retinal circuitry.

Disclosures: **J. Pottackal:** None. **J.H. Singer:** None. **J.B. Demb:** None.

Poster

225. Mechanisms of Retinal Circuit Assembly and Function

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CNPq(830608/1999-0)
CAPES

Title: Dendritic voltage-gated K+ currents stabilize rod-driven responses throughout growth on a computational model of a teleost mixed-input ON bipolar cell

Authors: *K. LEOPOLDO[1], M. KAMERMANS[2], C. JOSELEVITCH[1];
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Abstract: Retinal neurons are continuously formed in teleost species. Because rods are produced at a higher rate than second-order neurons, synaptic convergence changes significantly with growth in the teleost retina. In the goldfish retina, depolarizing bipolar cells that receive input from both rods and cones (ON mBCs) extend their dendrites laterally to accommodate a growing number of synapses with newly formed rods. We investigated how the addition of dendritic membrane, ionic channels and synapses impacts the integration of rod-driven signals by ON mBCs. We modeled a subtype of ON mBC (the mb1) using NEURON; biophysical and morphological data were gathered from literature. The model cell was synaptically connected to a variable number of photoreceptors and the model rods were hyperpolarized by voltage steps of increasing magnitude to analyze the effect of synaptic convergence onto ON mBC rod-driven responses. The ON mBC dendritic length was then gradually increased to study the effects of neuronal growth onto the integration of rod-driven signals by this neuron. Since real ON mBCs express voltage-gated K^+ channels at the dendrites, a delayed rectifier current (I_{KV}) was inserted at the dendritic tips of the model cell; the interaction of this voltage-gated conductance with the rod-driven input was subsequently investigated. Without I_{KV}, an 8-fold increase in rod convergence *depolarized* the ON mBC resting membrane potential (V_{rest}), whereas a 2-fold increase in dendritic length *hyperpolarized* V_{rest}. These changes in V_{rest} impacted the transmission of signals from rods to ON mBCs: dendritic growth *increased* ON mBC response amplitudes, whereas large rod convergence *decreased* response amplitudes. The addition of new rod synapses, but not dendritic growth, increased the sensitivity of ON mBCs to rod stimulation. On the other hand, dendritic growth led to a substantial decrease in response speed, due to the enlarged capacitance of the dendritic tree. Insertion of I_{KV} hyperpolarized ON mBC V_{rest} and

stabilized rod-driven response amplitudes and
sensitivity for all convergences and dendrite lengths tested. Activation of I_{KV} during rod stimulation also sped up ON mBC rod-driven responses and stabilized response time-to-peak throughout growth. These results suggest that (a) both dendritic growth and the degree of rod convergence influence ON mBC response amplitudes, sensitivity and speed and (b) dendritic I_{KV} stabilizes ON mBC responses throughout growth. Dendritic voltage-gated K^+ channels therefore act as a gain control mechanism in the rod-to-ON mBC synapse and enable ON mBCs to transmit a stable message to ganglion cells as the retina expands.

Disclosures: K. Leopoldo: None. **M. Kamermans:** None. **C. Joselevitch:** None.

Poster

225. Mechanisms of Retinal Circuit Assembly and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 225.18/L16

Topic: D.07. Vision

Support: NSERC
CIHR
FRQS

Title: Localization of transient receptor potential vanilloid type 1 in the monkey retina

Authors: *J. BOUSKILA[1], R. PALMOUR[2], J.-F. BOUCHARD[1], M. PTITO[1];
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Abstract: The presence of a widespread endocannabinoid system within the nervous system, including the monkey retina, has been demonstrated in recent years. Transient receptor potential vanilloid type 1 (TRPV1) is a cannabinoid-like non-selective ion channel that is present in the retina and binds to endocannabinoids, like anandamide and N-arachidonoyl dopamine. Expression patterns of TRPV1 are available for rodents and data in higher mammals like humans and monkeys are scarce. We therefore thoroughly examined the distribution pattern of TRPV1 throughout the retina of the vervet monkey (*Chlorocebus sabeus*) using immunohistochemistry and confocal microscopy. Our results demonstrate that TRPV1 is found mainly in the outer plexiform and inner plexiform layers, and in the retinal ganglion cell (RGC) layer. Co-immunolabeling of TRPV1 with parvalbumin, a primate horizontal cell marker, revealed a clear overlap of expression throughout the horizontal cell structure with most prominent staining in the cell body membrane and synaptic terminals. Furthermore, double label of TRPV1 and syntaxin was found throughout amacrine cells in the inner plexiform layer. Finally, double label of TRPV1 and RNA-Binding Protein With Multiple Splicing (RBPMS) allowed us to confirm its reported expression in the cell bodies and dendrites of RGCs. The presence of TRPV1 in the

lateral pathway suggests a function of this receptor in lateral inhibition, and thus contributes to enhance contrast and sharpness of visual stimuli.

Disclosures: **J. Bouskila:** None. **R. Palmour:** None. **J. Bouchard:** None. **M. Ptito:** None.

Poster

225. Mechanisms of Retinal Circuit Assembly and Function

Location: Hall A

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Program #/Poster #: 225.19/L17

Topic: D.07. Vision

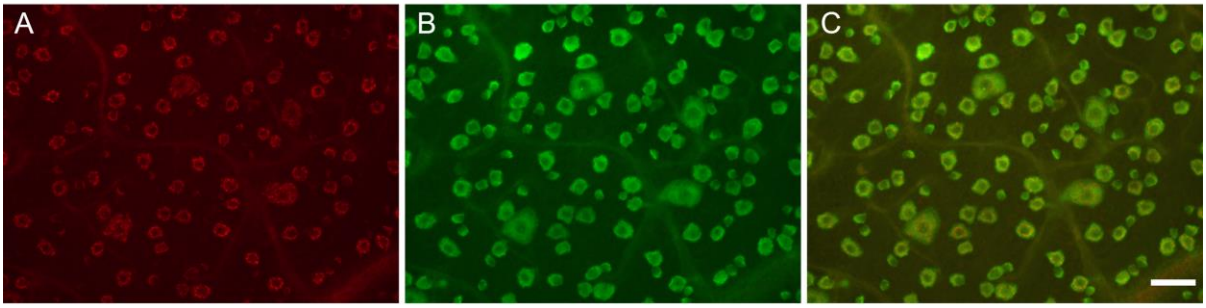
Support: National Science Foundation of China 31571091
National Basic Research Program of China 2015CB351806
China Postdoctoral Science Foundation 2017M620531

Title: Pannexin3 as a novel marker for ganglion cells in the feline retina

Authors: *W. WANG[1], Y. NAN[2], L. LUAN[2], J. GAO[2], K. DU[3], Y. TIAN[1], T. HUANG[1], M. PU[2];
[1]Peking Univ., Beijing, China; [2]Peking Univ. Hlth. Sci. Ctr., Beijing, China; [3]Karolinska Inst., Stockholm, Sweden

Abstract: Purpose: Identify Pannexin3 (Panx3) as an unique retinal ganglion cell (RGCs) marker.
Methods: Conventianl molecular biological techniques were applied to verify molecular properties of Panx3 protein. Morphological profiling and conventional immunohistochemistry were applied to RGCs in the young adult domestic cats. Panx3 antibody (Thermo Scientific Inc.) and RBPMS antibody (Pacific Immunology Inc.) were used to label RGCs. 4',6-Diamidino-2-Phenylindole, Dihydrochloride (DAPI, Roche Crop) was used to stain nuclei of retinal neurons. Soma size and topographic distribution pattern of these RGCs was investigated with a flurorescence microscope (Olympus BX-51) and analyzed with commercial software (Adobe Photoshop CS5 and Excel).
Results: The present study shows that Panx3 antibody selectively labeled RGCs (Panx3-RGCs) of various soma size at different retinal locations. The RBPMS served as a specific RGC marker in this experiment. The peak density of Panx3-RGCs was at the center of area centralis (AC) and the density reduced with eccentricities. To quantify density distribution patterns, the total number of Panx3-RGCs in each sampling area (333μm x 442μm, or 0.147mm^2) were counted at three eccentricities (1mm, 3mm, and 5mm). RGCs in four retinas were counted. The averaged density were 2282±336/mm^2 , 916±155/mm^2 , and 418±95/mm^2 , respectively. Next, Panx3 and RBPMS antibodies were used to double label RGCs. It was observed that, RBPMS-RGCs expressed Panx3 and vise versa. The averaged rate of double labeled cells reached 98 ±1.85 % at each

sampling area at the three eccentric locations.
Conclusions: Current investigation provides direct molecular and morphological evidence that Panx3 could be used as a selective RGC marker in the cat retina.



Disclosures: W. Wang: None. **Y. Nan:** None. **L. Luan:** None. **J. Gao:** None. **K. Du:** None. **Y. Tian:** None. **T. Huang:** None. **M. Pu:** None.

Poster

225. Mechanisms of Retinal Circuit Assembly and Function

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Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 225.20/L18

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Support: NSF 1557820
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IWU Hodson Research Institute

Title: Extracellular ATP-evoked signal transduction cascade underlying H+ efflux from retinal Müller glia

Authors: B. TCHERNOOKOVA[1], B. GOEGLEIN[2], T. LEUSCHNER[2], A. POWELL[2], A. SCHANTZ[2], R. P. MALCHOW[1], *M. A. KREITZER[2];
[1]Dept. Biol. Sci. & Ophthalmology, Univ. of Illinois at Chicago Dept. of Biol. Sci., Chicago, IL; [2]Biol., Indiana Wesleyan Univ., Marion, IN

Abstract: Adenosine 5'-triphosphate (ATP) is believed to be co-released with neurotransmitters and can act as a signaling molecule via the activation of ATP-sensitive ionotropic and metabotropic receptors. We have shown that low micromolar levels of extracellular ATP induce a significant increase in extracellular H+ flux from Müller cells isolated from the retinae of tiger salamanders and other vertebrates, including human. We have also previously shown that the ATP-induced acidification is mediated by activation of ATP-sensitive receptors and is dependent

on PLC-mediated increases of intracellular calcium from internal stores. In the present work we investigate the intracellular mechanisms underlying this ATP-receptor mediated extracellular acidification. Recordings of extracellular H^+ fluxes were obtained via the self-referencing technique and intracellular pH was monitored with BCECF. All recordings were performed with 1mM HEPES as the extracellular pH buffer with no bicarbonate added to the Ringer's solution. Stimulation of Müller glia with extracellular ATP leads to an intracellular acidification. The carbonic anhydrase inhibitors methazolamide and acetazolamide significantly reduce the extracellular H+ flux induced by ATP. These results are suggestive of mitochondria being a potential step in the ATP receptor-activated intracellular calcium signaling pathway. We hypothesize further that the stimulated mitochondria then produce CO2 that is converted to H+ and HCO3 via the enzyme carbonic anhydrase known to be present in Müller glial cells. A large fraction of the intracellular acid appears to be exported via a Na+/H+ exchanger, as the extracellular H+ flux induced by extracellular ATP is significantly reduced when extracellular sodium is removed or when Na+/H+ exchanger antagonists are added to the bath. Increased cytosolic calcium may also modulate the Na+/H+ exchangers' activity via interactions with intracellular proteins, such as calmodulin, which is suggested by a reduction in the ATP-induced extracellular acidification in preliminary studies with calmodulin antagonists. Our results highlight potential molecular mechanisms mediating the ATP-induced H+ flux from Müller glial cells. This ATP-mediated extracellular acidification may contribute to shaping signaling in the retina where extracellular pH has been shown to be an important modulator of neuronal activity.

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Poster

225. Mechanisms of Retinal Circuit Assembly and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 225.21/L19

Topic: D.07. Vision

Support: NIH Grant EY012141

Title: Glutamate transporters contribute to non-linear signaling and the mammalian cone photoreceptor synapse

Authors: *S. H. DEVRIES;
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Abstract: Glutamate transporters restrict transmitter spillover following synaptic release. This restriction often has little impact on the responses of postsynaptic receptors that directly oppose presynaptic release sites but can minimize the activation of extrasynaptic receptors. The

glutamate transporter EAAT5 is highly expressed by mammalian cone photoreceptors at their basal synaptic contacts with Off bipolar cells. We tested the idea that cone transporters located between transmitter release sites and glutamate receptors on postsynaptic dendrites can suppress transmission at low release rates establishing a threshold for signaling. Pairs of synaptically connected cones and Off bipolar cells were recorded in the whole cell configuration in slices from the cone dominant retina of the ground squirrel. Off bipolar cell type was identified via labeling with fluorescent dye. A cone was briefly (1 ms) stepped to a depolarized voltage of between -40 to -10 mV to evoke transmitter release. The epsc response was measured in the recorded bipolar cell held at -70 mV. At the same time, the glutamate transporter current of the releasing cone was measured and is an index of the overall amount of transmitter released. For Off bipolar cell types that make dendritic contacts close to ribbon vesicle fusion sites (ie, the cb2), the relationship between cone transmitter release and postsynaptic response was linear (n=8). For Off bipolar cell types that make basal terminal contacts with cones that are distant from transmitter release sites, the relationship between cone release and bipolar cell response was non-linear (power law exp=2.98±0.85, mean±SD, n=9). The non-linearity was such that small amounts of cone transmitter release failed to produce a response in the postsynaptic bipolar cell (eg, cb1a/b or cb3a/b types). Puffer application of TBOA (375 µm) increased the amplitude of synaptic responses at basal contacts (fold increase, cb1a: 2.38±1.41, n=9; cb3: 2.78±1.25, n=11) effectively linearizing the response, suggesting that transporter binding restricts access to these contacts. Studies on light responses confirm a functional impact of transporter binding in bright lights at low levels of cone glutamate release (n=5). By comparing the effects of transporter blockade on the different Off bipolar cell types, we conclude that transporters divide the Off bipolar cell types into two groups: cb2 cells are invaginating and have sensitive, low threshold responses, whereas the other 4 types make basal contacts near glutamate transporter binding sites and have a higher response threshold.

Disclosures: S.H. DeVries: None.

Poster

225. Mechanisms of Retinal Circuit Assembly and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 225.22/L20

Topic: D.07. Vision

Title: Electrophysiological and morphological properties of the retinal ganglion cell subtypes which project to amygdala

Authors: *D. GREER, G. SCHWARTZ;
NUIN, Northwestern Univ., Chicago, IL

Abstract: We used an established retrograde tracing method to discover the retinal ganglion cell (RGC) subtypes projecting to the medial amygdala through injecting a virus (AAV1) containing Cre recombinase into the retino-recipient sub-region. We used RGC classifications established in our lab and previous literature, including Eyewire, to estimate the proportion of input from each RGC type after characterizing based on morphological and electrophysiological properties. We also looked for interactions between retinotopy and functional classification, such as projections from a certain RGC type confined to the ipsilateral or contralateral eye or to a certain portion of the visual field.

Disclosures: D. Greer: None. **G. Schwartz:** None.

Poster

225. Mechanisms of Retinal Circuit Assembly and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 225.23/DP09/L21

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: D.07. Vision

Title: A layered retina structure to study neuronal circuits in human stem cell-derived organoids

Authors: *M. RIZZI, K. POWELL, M. BRANCH, M. BASCHE, E. LANNING, M. KLOC, R. MASWOOD, A. GONZALEZ-CORDERO, E. WEST, A. SMITH, R. ALI;
UCL, London, United Kingdom

Abstract: The study of human neuronal circuits currently relies on the availability of post-mortem tissue (Beaulieu-Laroche et al., 2018). Human stem cell-derived organoids offer an exciting alternative to this approach, providing more reliable sources of human neural tissue (Gonzalez-Cordero et al., 2013; Lancaster et al., 2013; Quadrato et al., 2017), including at different developmental stages and from subjects with specific relevant genetic mutations (Clevers, 2016). Although it is now possible to differentiate organoids containing cell types from regions of the nervous system (e.g.: retina, hindbrain, forebrain), neural circuit function relies on a finely regulated anatomical structure and connectivity. We therefore focused on improving the circuit architecture, in order to provide a reliable model for functional studies. The human retina is highly suited to measure such improvements, given its hardwired, layered circuitry (reviewed in Masland, 2012). Here we identified optimized neuronal differentiation conditions for both mouse and human stem cell derived organoids. In particular, for human organoids, we are able to differentiate layered retinal structures, which contain the main neuronal cell types required for retina computation, including bipolar cells, subtypes of amacrine cells and ganglion cells, as well as well developed photoreceptor cells. Using a variety of imaging techniques, we find that the improved culturing conditions allow the formation of an Outer Plexiform Layer, where the first

microcircuitry of the visual system resides, which is better-structured than with previously published methods. We also measured neuronal activity in both mouse and human stem cell derived layered retinal structures by using GCaMP6 and iGluSnFr sensors. For both types of culture, we find spontaneous activity that resembles that normally observed in developing retinas. Our approach and optimization provide a viable solution to the generation of large numbers of retinal structures that will allow the study of function and computation in retinal circuits that are either healthy or affected by specific congenital forms of degeneration.

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Poster

225. Mechanisms of Retinal Circuit Assembly and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 225.24/L22

Topic: D.07. Vision

Support: EY029719

Title: Glycine receptor subunit expression in mouse ON-OFF direction selective retinal ganglion cells

Authors: *I. S. PYLE[1], **M. A. MCCALL**[2];
[1]Anatom. Sci. and Neurobio., [2]Ophthalmology & Visual Sci., Univ. of Louisville, Louisville, KY

Abstract: Retinal ganglion cells (RGCs) encode the visual scene, and their response is a consequence of integrated excitatory and inhibitory inputs from the upstream retinal circuit. Retinal inhibition is mediated by γ-aminobutyric acid and glycine receptors (GlyRs). Four GlyR isoforms result from the combination of a single beta subunit with one of four alpha subunits (1 – 4) in a stoichiometry of 3β2α. The α subunits are: differentially expressed across the inner retina, highly conserved across species and control the decay kinetics of the spontaneous inhibitory postsynaptic currents (sIPSCs). GlyRα1 sIPSCs have fast decays (3 msec), GlyRα3 sIPSCs are slower (9 msec), and GlyRα2 and GlyRα4 have similar, very slow decays (20 msec). The kinetics, along with the properties of presynaptic glycine release likely enhance glycinergic inhibitory properties throughout the retina.Among the ~40 types of RGCs are cells sensitive to the direction of stimulus (DS) motion. A subset of these DS RGCs respond to both light onset, and light offset (ON/OFF) and are named ON/OFF DS (ooDS) RGCs. In these RGCs, GABAergic inhibitory input establishes direction selectivity. In the absence of glycinergic inhibition (Strychnine), direction selectivity encoded in ooDS spiking responses is unaffected and it is unclear if ooDS GCs express GlyRs.We examined the synaptic inputs to ooDS RGCs by

recording their sIPSCs (whole cell patch clamp). We verified the presence of both large amplitude, slow GABAergic sIPSCs, and glycinergic sIPSCs. The decay kinetics of the glycinergic sIPSCs are slow, which predicts they express either GlyRα4 or GlyRα2. To test our idea, we crossed ooDS fluorescent reporter mice to GlyRα4 or GlyRα2 global KO mice. In ooDS RGCs in either single KO mice, the frequency of glycinergic sIPSCs does not change, whereas, in GlyRα4/GlyRα2 double KO ooDS RGCs, no glycinergic sIPSCs remain. We suspected a compensatory mechanism of some kind could occur and tested this idea by knocking down either GlyRα4 or GlyRα2 in RGCs, using a retrogradely transported rAAV shRNA injected into the superior colliculus. In rAAV shRNA infected ooDS RGCs, sIPSC frequency was reduced when either GlyRα4 or GlyRα2 expression was knocked down. We conclude that ooDS RGCs receive synaptic glycinergic inputs, mediated by both GlyRα2 and GlyRα4. These results lead the way toward understanding how synaptic inputs from two different GlyRs can shape the visual signaling of ooDS RGCs.

Disclosures: I.S. Pyle: None. **M.A. McCall:** None.

Poster

225. Mechanisms of Retinal Circuit Assembly and Function

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 225.25/L23

Topic: D.07. Vision

Title: Anoctamin 1: A potential partner for CFTR-mediated nitric oxide-induced chloride release in retinal amacrine cells

Authors: *T. RODRIGUEZ[1], L. ZHONG[2], A. RADER[2], H. SIMPSON[2], E. L. GLEASON[3]; [1]LSU Biol. Sci., Baton Rouge, LA; [2]Louisiana State Univ., Baton Rouge, LA; [3]Biol. Sci., LSU, Baton Rouge, LA

Abstract: In chick retinal amacrine cells (ACs), the sign of GABAergic synaptic transmission is regulated by a nitric oxide (NO)- and cystic fibrosis transmembrane conductance regulator (CFTR)-dependent mechanism that releases Cl^- from internal stores. The anoctamin (ANO) family of transmembrane proteins is functionally diverse. ANOs 1/2 are verified Cl^- channels while others have more ambiguous function, some are lipid scramblases (ANO6) or nonselective ion channels. Recent reports suggest the presence of either ANO1 or ANO6 is necessary for cAMP- activated CFTR-dependent Cl^- secretion in epithelial cells (Benedetto et al. 2019). Because NO also generates a cytosolic Ca^{2+} signal in ACs, we explored the involvement of the Ca^{2+}-activated Cl^- channels ANO1 in the NO-dependent Cl^- release. Two pieces of evidence suggest that ANO1 is involved in the effects of NO. First, ACs loaded with the Ca^{2+} chelator BAPTA do not respond to NO with elevated cytosolic Cl^-. Second, an ANO1/2 inhibitor (T16Ainh-A01) suppresses this same effect of NO. To further explore the involvement ANO1,

The expression of ANO family members was analyzed in mixed retinal cultures using RT-PCR. *ANOs1-6* and *ANOs8-10* were amplified and PCR products were verified by sequencing. The expression of *ANO1* specifically in ACs was verified using single-cell RT-PCR. To assess ANO1 protein expression, a rabbit monoclonal antibody specific for human ANO1 (Abcam ab190803) was selected based on epitope sequence similarity to chicken ANO1. The specificity of this antibody in chicken tissue was evaluated using Western blot analysis and cloud point extraction. A single band at the predicted molecular mass of ANO1, 117KDa, was present in non-reducing conditions when separated by SDS-PAGE and blotted to a nitrocellulose membrane. Cloud point extraction by Triton X-114 revealed presence of the protein in both the aqueous and detergent rich phases consistent with other channel-forming integral membrane proteins. Immunocytochemistry using the same ANO1 antibody shows punctate labeling in both cell bodies and processes of ACs. In some ACs, labeling was especially heavy in primary processes and out in growth cones. Labeling was also concentrated at points of contact between processes which can be sites of synaptic contact. Cone cells and cells with small cell bodies (<10 µm dia., tentatively identified as bipolar cells) were also labeled. These results support the expression of ANO1 in ACs giving these channels the potential to collaborate with CFTR to mediate the NO (and Ca^{2+})-dependent release of internal Cl^{-}.

Disclosures: **T. Rodriguez:** None. **L. Zhong:** None. **A. Rader:** None. **H. Simpson:** None. **E.L. Gleason:** None.

Poster

225. Mechanisms of Retinal Circuit Assembly and Function

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Program #/Poster #: 225.26/L24

Topic: D.07. Vision

Support: National Nature Science Foundation of China (81570842)

Title: Melanopsin-containing subpopulation analysis of retinal ganglion cells in culture

Authors: *S. WU, X. MO;
Eye, Ear, Nose & Throat Hosp. of Fudan Univ., Shanghai, China

Abstract: Purpose: To Characterize the proportion of melanopsin-containing retinal ganglion cells (mRGCs) among the entire cultured RGC population.Methods: Postnatal mouse RGCs were isolated, purified, cultured and fixed at different timepoints, followed by immunofluorescent staining using anti-Brn3a, Melanopsin, and Neurofilament H primary antibodies as well as Hoechst 33342 counterstaining. Cell survival were determined and quantified by Brn3a expression and nuclear morphology. Also, Melanopsin+ and/or Neurofilament H+ RGC proportion were quantified under fluorescence microscopy.Results: Over 98% of surviving

RGCs were Melanopsin+, and 80% of Melanopsin+ RGCs were Neurofilament H+. No significant proportion differences of Melanopsin+ or Neurofilament H+ RGCs were found between postnatal Day 1 and Day 5 RGCs or among different timepoints.Conclusion: Considering the high proportion of mRGC in cultured RGCs, our results provide a better understating on subtypes of RGCs in culture. Also, these data suggest that RGCs melanopsin-deficient RGCs may receive melanopsin from mRGCs, which may be a survival-promoting approach, in culture.

Disclosures: S. Wu: None. **X. Mo:** None.

Poster

225. Mechanisms of Retinal Circuit Assembly and Function

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Swiss National Science Foundation Ambizione Grant PZ00P3_167989
Swiss SystemsX interdisciplinary PhD grant “Systems biology of vision: Identification of visual coding properties of retinal ganglion cells"

Title: Improved receptive-field characterization of retinal ganglion cells using a large field-of-view light stimuli

Authors: *R. DIGGELMANN, M. ŽNIDARIč, A. BUCCI, A. HIERLEMANN, F. FRANKE; D-BSSE, ETH Zürich, Basel, Switzerland

Abstract: The retina is an ideal model to study sensory processing, as it is possible to conduct *ex vivo* experiments, in which the input in the form of light stimulation can be precisely controlled and the output in the form of retinal ganglion cell (RGC) activity can be recorded. It is estimated that there are more than 30 RGC types that encode different aspects of visual information; the information is then sent through parallel pathways to the brain. An important feature of each RGC is the spatiotemporal receptive field (RF). Its spatial component represents the area in the visual scene to which an RGC is responsive, while its temporal component represents its preferred contrast kinetics. RFs can be understood as linear filters in a stimulus-response model. Under this assumption, simultaneous characterization of large numbers of RFs is typically performed by using reverse correlation of RGC responses to a white-noise stimulus, i.e. a fast succession of random checkerboard patterns. However, many RGC types have highly non-linear responses. Therefore, they do not respond well to this stimulus for a number of reasons, including wide-field suppression, low contrast in the RF area and lack of locally coherent

motion, which is characteristic of a natural visual scene. Stimuli that are better suited to elicit non-linear RF properties were generally designed to characterize single cells. Modern technologies, such as calcium imaging or high-density microelectrode arrays (HD-MEAs), enable us to record action potentials from hundreds of RGCs simultaneously over an area spanning several square millimeters. Under these conditions, traditional stimuli cannot be used to characterize all cells within a limited lifetime of an explanted retina, so that new approaches are necessary. Here we present light stimuli comprising a multitude of objects that move independently across the visual scene. The parameters of each object, including size, contrast, speed and direction, are randomly distributed, so that the entire visual scene is uniformly covered over the duration of the stimulus. Our new stimuli offer several advantages: they probe all recorded RGCs simultaneously, covering a large field-of-view; they contain locally coherent motion; they are relatively simple to analyze; they reveal RF kinetics and they can be used to infer non-linear features, such as direction selectivity. We show that we can identify RF locations of up to 3 times more RGCs with such a 'random-moving-object' stimulus than with a standard white-noise stimulus in the same time span. We further present ways to extract additional features of the RFs, such as polarity, contrast kinetics and direction selectivity.

Disclosures: R. Diggelmann: None. **M. Žnidarič:** None. **A. Bucci:** None. **A. Hierlemann:** None. **F. Franke:** None.

Poster

225. Mechanisms of Retinal Circuit Assembly and Function

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Support: Swiss National Science Foundation Sinergia Project CRSII5_173728
Swiss National Science Foundation Ambizione grant PZ00P3_167989 [F.F.]
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Title: How the coupling strength of horizontal cells effects the retinal processing of spatio-temporal light stimuli - Model and experiments

Authors: *A. BUCCI[1], R. DIGGELMANN[1], M. ŽNIDARIč[1], B. ROSKA[2], R. DA SILVEIRA[3], A. HIERLEMANN[1], F. FRANKE[1];
[1]D-BSSE, ETH Zürich, Basel, Switzerland; [2]IOB, Basel, Switzerland; [3]ENS, Paris, France

Abstract: In the retina, photoreceptors (PRs) convert an input of light into electrical signals that eventually result in an output of spikes in ~30 types of retinal ganglion cells (RGCs). Natural images falling on the retina contain strong spatial correlations; therefore, PR activity is highly redundant. Lateral processing pools this redundant electrical activity in order to generate RGC

spiking activity. The synapse between PRs, bipolar cells and horizontal cells (HCs) is the first location in the visual processing hierarchy where lateral processing occurs. Bipolar cells pool their input from multiple PRs and pass on the signal via parallel pathways to the RGCs. HCs (i) receive excitatory input from PRs, (ii) are strongly laterally coupled by gap junctions, and (iii) feed spatially averaged inhibitory signals back to the PRs. In this work, we combined theoretical and experimental approaches to investigate how HCs participate in the retinal processing of light stimuli that are not spatially uniform. We developed a model consisting of coupled differential equations, which describe the dynamics of interactions between PRs and HCs. In the model, PRs independently detect light levels, but are laterally connected to each other via HCs. The lateral connections between HCs are weighted by a coupling strength parameter that controls the lateral spread of electrical activity. We analyzed how the spiking activity of different model RGCs are affected by (i) removing HC feedback to the PRs and (ii) varying the coupling strength between HCs. Experimentally, we measured how HC feedback to the PRs shapes the spiking activity of RGCs by specifically and reversibly perturbing the activity of HCs. To do so, we used chemogenetics in ex-vivo experiments with mouse retinae. We monitored changes in the light-induced spiking activity of the very same RGCs before, during and after the perturbation by means of high-density microelectrode arrays. With our model, we elucidated the effect of lateral connectivity of HCs on neighboring PRs that are not directly excited, but still participate in visual processing. Ultimately, combining the approaches will help us to better understand the function of HCs in the presence of stimuli that are not spatially uniform.

Disclosures: **A. Bucci:** None. **R. Diggelmann:** None. **M. Žnidarič:** None. **B. Roska:** None. **R. da Silveira:** None. **A. Hierlemann:** None. **F. Franke:** None.

Poster

225. Mechanisms of Retinal Circuit Assembly and Function

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Title: *Ex vivo* mouse retinal recordings using HD-MEA systems: From characterizing single cells to analyzing populations

Authors: ***M. ŽNIDARIč**, R. DIGGELMANN, A. BUCCI, A. HIERLEMANN, F. FRANKE; D-BSSE, ETH Zürich, Basel, Switzerland

Abstract: *Ex vivo* retinae have been a neuroscientific model of choice since the 1960s. They represent an ideal input-output model, where one can, through light stimulation, precisely control the input and, at the same time, record the output in the form of retinal-ganglion-cell (RGC) activity. Initially, neuroscientists were able to record action potentials from a single or, at best, a few RGCs at once. This number has increased significantly over the last decades. With the availability of methods like calcium imaging and large-scale high-density microelectrode arrays (HD-MEAs), it is possible to monitor the activity of hundreds and, in some cases, even thousands of RGCs simultaneously. This technological leap not only increases the quantity of available data but also opens the possibility to work on a number of previously nonaddressable scientific questions, such as whole-RGC-population computations or topographic differences in retinal processing. Recent characterizations of large populations of identified RGC types in zebrafish [Zimmermann et al. 2018] and mice [Warwick et al. 2018, Sabbah et al. 2017] demonstrated that cells of a single type show functional differences depending on their spatial position within the retina. This finding emphasizes the need to not only record from large populations of RGCs but to also precisely track the original RGC position and orientation of the population within the intact eye. It remains a challenge to precisely preserve the original orientation of the *ex vivo* retina piece during experiments with HD-MEAs. Moreover, a number of technical and computational barriers of large-scale RGC recordings have to be overcome, as manual analysis and filtering methods are no more applicable. Here, we present an HD-MEA recording setup that enables high signal-to-noise measurements of RGC activity covering an area of up to ~4.5 mm^2, or approximately 90 degrees of the mouse visual space. We demonstrate simultaneous extracellular recordings and light stimulation of hundreds of RGCs extending over large visual distances while maintaining a good estimate of their locations within the intact eye. Finally, we debate the challenges of establishing a high-throughput workflow for stimulation and automated analysis of signals of large populations of RGCs in mouse *ex vivo* retinae.

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Poster

226. Eye Movements and Perception

Location: Hall A

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Program #/Poster #: 226.01/L28

Topic: D.08. Visual Sensory-motor Processing

Support: Canadian Institutes of Health Research (FRN 148365)
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Title: Task-based fMRI of the visuomotor network in the marmoset monkey: Comparison with human visuomotor network topology

Authors: *D. J. SCHAEFFER, K. GILBERT, Y. HORI, L. HAYRYNEN, K. D. JOHNSTON, J. GATI, R. S. MENON, S. EVERLING;
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Abstract: Visuomotor tasks are often used to index aberrations of cognitive function in patient populations, with several neuropsychiatric and neurologic disorders characterized by visuomotor dysfunction. In order to probe the etiology of the deficits preclinically, understanding how human visuomotor network topologies conform across non-human primate models is invaluable. The common marmoset (*Callithrix jacchus*) has received recent attention as a powerful model in the visual neurosciences - marmosets are amenable to a host of genetic manipulation techniques and have a lissencephalic cortex, which is well suited for a variety of recording techniques (e.g., calcium imaging, laminar electrophysiology). Because the marmoset cortex is mostly lissencephalic, however, the locations of frontal visuomotor regions (e.g., frontal eye fields (FEF)) are less readily identified than in Old World macaque monkeys. Further, although high quality histology-based atlases do exist for marmosets, identifying these regions based on histology alone is not always accurate, with the cytoarchitechtonic boundaries often inconsonant with functional boundaries. As such, there is a need to map the functional location of these regions directly. Task-based functional magnetic resonance imaging (fMRI) is of utility in this regard, allowing for detection of whole-brain signal changes in response to visuomotor stimuli. Here, we conducted ultra-high field task-based fMRI in both marmosets (at 9.4 T) and humans (at 7 T), with the purpose of comparing visuomotor network topologies between the two species. Using a surface-based mapping transformation, we demonstrate homologous networks between the two species, both with peaks in primary visual cortex, area V6, lateral intraparietal area, frontal eye fields and middle cingulate regions. Overall, these results support the view that marmosets are a promising preclinical modelling species for studying visuomotor dysfunction related to neuropsychiatric or neurodegenerative human brain diseases.

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Poster

226. Eye Movements and Perception

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Topic: D.08. Visual Sensory-motor Processing

Support: Canadian Institutes of Health Research (FRN 148365)
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Title: Visual looming and receding stimuli activate a large brain network in the common marmoset

Authors: *J. C. CLÉRY[1], D. J. SCHAEFFER[1], Y. HORI[1], K. M. GILBERT[1], J. S. GATI[1], R. S. MENON[1], S. EVERLING[1,2];
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Abstract: The common marmoset (*Callithrix jacchus*) is a small-bodied New World primate that has been recently identified as a powerful model to study brain functions in addition to canonical Old World macaque monkeys. Its lissencephalic cortex allows access to many cortical regions for electrophysiological or neuroimaging technics, thus making marmosets a potentially powerful nonhuman primate model for the study of complex visual processing.
Here we used functional magnetic resonance imaging (fMRI) to explore responses to looming visual stimuli in marmosets which are known to activate large networks in macaques and humans. We performed fMRI on awake marmosets in a 9.4T scanner by using visual stimuli either looming toward the animals or receding away from them. Both types of visual stimuli evoked large brain activations across the brain with strong activations in visual, temporal, parietal and frontal areas. However, looming stimuli elicited not only a more widespread network but also activated more specifically temporal areas (TE2-3/FST), frontal areas (area 12/45), parietal area (MIP/VIP), visual areas (V3a/V4) as well as subcortical areas (Thalamus, caudate, superior colliculus).
Interestingly, the majority of these activations is also found in the macaque brain when visual looming stimuli predicted a tactile stimulus (Cléry et al. 2017) highlighting an alert network. This suggest that even in absence of potential tactile stimulation, the marmoset brain is ready to treat any potential threat or impact and that this network is shared between primate species.

Disclosures: **J.C. Cléry:** A. Employment/Salary (full or part-time):; BrainsCAN postdoctoral fellowship (CFREF). 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.; Canadian Institutes of Health Research (FRN 148365). **D.J. Schaeffer:** None. **Y. Hori:** None. **K.M. Gilbert:** None. **J.S. Gati:** None. **R.S. Menon:** None. **S. Everling:** None.

Poster

226. Eye Movements and Perception

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Topic: D.08. Visual Sensory-motor Processing

Support: NIH T32 EY007125
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Title: Neuronal mechanisms of pre-saccadic attention in middle temporal area of the marmoset monkey

Authors: *S. H. COOP, J. L. YATES, J. F. MITCHELL;
Brain and Cognitive Sci., Univ. of Rochester, Rochester, NY

Abstract: We move our eyes 2-3 times per second to bring the high-resolution, central part of our vision (the fovea) onto objects of interest. Each eye movement produces perceptual enhancements before the eyes move, including a sharpening of tuning for target features (Li, Barbot, and Carrasco, 2016). Neurophysiological studies in the macaque monkey have shown a related enhancement of neural sensitivity in extra-striate areas prior to eye movements into the receptive field (Moore and Chang, 2009). However, it remains unknown how the perceptual tuning enhancements for stimulus features are produced at the neuronal level. We thus examined how neuronal tuning curves for motion direction in the middle temporal area (MT) of the marmoset monkey changed before saccades into the receptive field. Marmosets made a saccade from central fixation to one of several equally eccentric stimuli (full coherence motion dot fields). Any target location was rewarded as long as it was not selected in the previous trial. We recorded from MT neurons as a saccade was planned towards or away from the receptive field. We measured the responses of single units across 16 directions of motion during the 50-100 ms preceding the saccade. Tuning curves in the saccade towards and away conditions were fit with an adjusted Von Mises function that allowed us to isolate additive and multiplicative effects of saccades from changes in the shape of the tuning curves. In a single monkey, we find that most neurons exhibit additive and gain increases, with a small number also showing sharpening in tuning. Overall, the changes in tuning are comparable to those classically observed in paradigms of covert attention (McAdams and Maunsell, 1999; Treue and Martinez-Trujillo, 1999). We further examined if single neurons improved their sensitivity using a receiver-operator characteristic (ROC) analysis. Consistent with a previous study in macaque V4 (Moore and Chang, 2009), we find that many individual neurons show an increase in sensitivity for the saccade target. Taken together, these results show first that marmosets exhibit comparable pre-saccadic enhancements as macaques, and second, that changes in pre-saccadic neuronal tuning are comparable to those observed in classic covert attention paradigms.

Disclosures: S.H. Coop: None. **J.L. Yates:** None. **J.F. Mitchell:** None.

Poster

226. Eye Movements and Perception

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Topic: D.08. Visual Sensory-motor Processing

Support: JSPS KAKENHI JP19H04993

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JSPS KAKENHI JP17H06039
JSPS KAKENHI JP19K20653

Title: Cerebral information dynamics from visual input to motor output with a whole-hemisphere electrocorticography (ECoG)

Authors: *T. KANEKO[1,2], M. KOMATSU[1,3], N. ICHINOHE[1,3], H. OKANO[2,1];
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Abstract: Advances in neuroscience has accumulated a snapshot understanding on how eye-movement is generated or how object identity is analyzed in particular neural structures. However, it remains unknown how spatial and temporal interactions of multiple cortical areas generate sequences of active visual behavior under naturalistic condition. In the present study, we report the cortical information dynamics of visual behavior by recording electrocorticographic (ECoG) signal covering the entire hemisphere of a marmoset brain while the marmoset freely viewed naturalistic movie stimuli. We found that, under natural visual behavior, visual computation did not start from primary visual cortex rather MT complex and posterior parietal cortices which showed distinct pattern of efferent and re-afferent activity just after saccade onset. Furthermore, we showed that express post saccade activities transmitted to, but not from, the early visual areas. We also found that information is not continuous flow even though retina can sample visual information continuously, but rather a packet of information which travels from dorsal to ventral visual stream. Furthermore, we found active vision was intrinsically recurrent process where brain and behavior coordinated to maintain certain amount of neural activity, that is saccade triggered a transient neural activity and the following saccade occurred to refresh neural activity just before sum of a whole brain activity became a silent. Here we described several novel features of macro-level information dynamics which might be crucial for efficient and stable perception of natural active visual behavior. Understanding brain dynamics under natural vision may contribute, not only understanding how visual system is designed to work on real-life problems, but also development of a new AI architecture which requires time-efficient on-time computation such as for robotics.

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Poster

226. Eye Movements and Perception

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Topic: D.08. Visual Sensory-motor Processing

Support: NIH R01EY029788-01
NSF1534932

Title: Modulations of visual perception across the foveola

Authors: *M. POLETTI, N. SHELCHKOVA;
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Abstract: The foveola, the retinal region responsible for fine spatial vision, covers less than 0.1% of the visual field. We previously showed that fine spatial vision deteriorates at larger eccentricities across this 1-deg region. Here we examine how visual sensitivity is characterized along isoeccentric foveal locations just ~7 arcminutes away from each other. To this end, we relied on a combination of techniques allowing for high-resolution recordings of eye position and accurate gaze localization.

Observers (n=4) fixated on a marker surrounded by eight boxes (5'x5') arranged in a circle (20' radius). Observers were instructed to maintain their gaze at the center of the array throughout the trial. Then, nine high-acuity probes (7'x2' bars) were briefly flashed one in each box, and one at the center of gaze. After a blank period, a response cue appeared. Subjects reported the orientation of the probe previously presented at the location indicated by this cue. Performance was assessed at each probe location. To eliminate confounding factors associated with eye movements, we selected for analysis only trials without microsaccades, in which subjects maintained tight fixation on the central marker.

Our findings show that sensitivity to fine details at isoeccentric foveal locations is not uniform. Interestingly, each subject shows an idiosyncratic distribution of sensitivity. Overall, sensitivity was highest nasally (average performance increment at nasal locations compared to the other locations: 24%, $p<0.03$). At the locations characterized by highest sensitivity response times were consistently lower (average difference 87 ms, $p<0.03$). These results in part resemble what happens at much larger eccentricities across the visual field; it is known that sensitivity is better along the horizontal than the vertical meridian. However, differently from what happens across the rest of the visual field, we do not report a difference in sensitivity between upper and lower meridian at the foveal scale.

These findings shed new light on how stimuli are processed in the foveola, and on how visual processing at this scale differs from the rest of the visual field. At the level of the retina, cone density is known to be highest along the horizontal meridian. However, it is not clear whether a similar asymmetry is present also at the foveal scale. Therefore, it remains to be determined whether the reported asymmetries in foveal sensitivity are linked to differences in the packing of cone photoreceptors at isoeccentric locations across the foveola, or if they arise from differences in the cortical processing of isoeccentric foveal stimuli.

Disclosures: M. Poletti: None. **N. Shelchkova:** None.

Poster

226. Eye Movements and Perception

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Topic: D.08. Visual Sensory-motor Processing

Support: NIH Grant R01-EY025648

Title: Decoding 3D spatial location across saccades in human visual cortex

Authors: *X. ZHANG, C. M. JONES, J. D. GOLOMB;
Dept of Psychology, Ohio State Univ., Columbus, OH

Abstract: Visual signals are initially processed as two-dimensional images on our retina. To perceive a 3D world, depth information must be reconstructed from these 2D images. In a previous study from our lab, we explored how different parts of human visual cortex represent 2D spatial location and position-in-depth, with visual stimuli presented during stable fixation (Finlayson, Zhang & Golomb, 2017). We found that along the visual hierarchy, spatial representations gradually transitioned from 2D-dominant to balanced 3D. However, in daily life we make frequent eye movements, and consequently the 2D retinal inputs constantly change. With these dynamic inputs, are the neural representations of depth information robust across saccades? In the current study, we investigate how position-in-depth is represented in the brain across saccades compared to during sustained fixations. In an fMRI scanner, while wearing red-green anaglyph glasses, participants passively viewed a random dot patch that appeared randomly in one of four 3D screen locations in blocks of 16s duration. Each location was defined by its 2D position (above or below screen center; y-axis information), and its depth position (in front or behind screen center; z-axis information). In half of the blocks, subjects maintained fixation on a stable fixation dot throughout the block (fixate left and fixate right conditions). In the other blocks, the fixation dot repeatedly alternated between the left and right sides of the screen to trigger saccades (saccade condition). Multivariate pattern analysis (MVPA) was used to assess the brain representations of 2D location and position-in-depth information in the fixation and saccade conditions. In the fixation conditions, we replicated the pattern of results found in the previous study, that both 2D (y) information and depth (z) information could be decoded in intermediate and later visual areas. However, both 2D (y) and depth (z) information was highly dependent on eye position: we found little information that could be decoded *across* different eye positions in any visual ROIs. Interestingly, in the saccade condition, we could decode both types of information in several ROIs, with a decrease of y information and an increase of z information along the visual hierarchy. These results show that the representations of 3D spatial location were dependent on eye position during sustained fixations, but when participants made repetitive

saccades, the depth representation could still be decoded. This might indicate that depth information is, to some extent, tolerant of saccades in later visual areas.

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Poster

226. Eye Movements and Perception

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 226.07/L34

Topic: D.08. Visual Sensory-motor Processing

Title: Bottom-up attention in association with microsaccade rate

Authors: *S. NOGUCHI[1], T. KOHAMA[2], H. YOSHIDA[3];
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Abstract: Focusing or reallocating attention while actively fixating on the visual stimuli modulates the rate of microsaccades, which are involuntary jumps in visual fixation (Rolfs 2009). The microsaccade rate shows rebound after inhibition in response to the abrupt onset of the cue, indicating that the occurrence of microsaccades explains the state of covert attention (Laubrock et al. 2005). However, little is known about the attentional modulation of the microsaccade rate in passive viewing. In this study, to investigate the properties of microsaccades induced by bottom-up attention, we conducted an experiment in which the subjects maintained visual fixation during passive viewing of randomly presented peripheral spotlights. The subjects were 6 college students who were instructed to maintain visual fixation on a crosshair pattern presented at the center of a liquid crystal display screen and to passively view the peripherally displayed spotlight targets. The number of targets to be presented in each trial was randomly decided between 3 and 5. The target location was selected from 1 of 8 directions (0, 45, 90, 135, 180, 225, 270, or 315 deg) and 1 of 2 eccentricities (4 or 8 deg). The brightness of the target in each experimental block was set to 4 intensity levels for each subject based on the discrimination threshold determined from the preliminary experiments: "far upper" (FU), "near upper" (NU), "near lower" (NL), and "far lower" (FL). Microsaccades were detected using an order-statistic time-window analysis (Ohtani et al. 2016), and the transitions of microsaccade frequencies associated with the onset of the target were analyzed. The results showed that around 300 ms after the onset of the target, transient inhibition and rebound responses of the occurrence of microsaccades were observed in FU, in which the subjects detected the targets satisfactorily. In contrast, these responses completely disappeared in FL, in which the target was almost invisible. This suggested that the existence of the rebound after inhibition of microsaccade occurrence showed the bottom-up attentional modulation of microsaccade generation and reflected the passive perception of peripheral visual events.

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Poster

226. Eye Movements and Perception

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Topic: D.08. Visual Sensory-motor Processing

Support: NIH NEI EY07977
NIH NEI EY018363

Title: Modeling the trial-by-trial influence of fixational eye movements on visual discrimination

Authors: ***Y.-C. LIN**[1], M. RUCCI[3], J. D. VICTOR[2];
[2]Feil Family Brain and Mind Res. Inst., [1]Weill Cornell Med. Col., New York, NY; [3]Boston Univ., Boston, MA

Abstract: Our eyes move constantly, even during the periods of fixation between saccadic shifts of gaze. While the approximately Brownian nature of these fixational eye movements (FEM) suggests that they merely reflect oculomotor noise, it is now known that the temporal modulations they produce are an important computational step for vision, and that they are under neural control. Such neural control would enable FEM trajectories to adapt to the visual task. To determine the consequences of changes in FEM, we developed a model that enables probing the effects of individual FEM trajectories on high-acuity visual performance.
The model had two inputs: visual stimuli, consisting of targets such as optotypes with superimposed noise, and FEM trajectories, measured during simple visual discrimination tasks or simulated by random walks. Neural activity, represented as time-varying firing rates, were determined by transforming the moving retinal image by spatiotemporal filters whose parameters were determined from receptive fields of macaque retinal ganglion cells and lateral geniculate nucleus neurons. Behavioral responses were derived from the neural activity via a Bayesian decision stage. For each neuron, Fisher discriminant analysis identified the optimal linear discriminator that distinguished each possible target from all the others. This yielded a likelihood ratio for each possible target, each FEM trajectory, and each neuron. To pool information across neurons, we assumed conditional independence. The behavioral response was then determined by the maximum a posterior probability. Finally, a confusion matrix and fraction correct is determined for each FEM trajectory.
The model allows for probing the influences of FEMs in many ways. First, it tests the extent to which different eye movement trajectories influence discrimination performance. We hypothesize that, for a given visual discrimination, the FEM trajectories that generate the largest luminance transients have the greatest impact on performance. To understand the roles of different classes of LGN neurons in making use of FEM dynamics, we can selectively model

magnocellular vs. parvocellular neurons, or ON vs. OFF neurons, and examine effects on performance. Since the model predicts effects of individual FEM trajectories on performance, it can be used in conjunction with subjects' error patterns to test individual sensitivity to FEM-modulated visual signals. In sum, the model formalizes a link between fixational eye movements, neural activity, and behavioral responses, and is thus a general approach for understanding how the dynamics of FEMs encode spatial information.

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Poster

226. Eye Movements and Perception

Location: Hall A

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Program #/Poster #: 226.09/L36

Topic: D.08. Visual Sensory-motor Processing

Title: Task irrelevant visual forms facilitate saccadic eye movements

Authors: *A. R. BOGADHI, Z. M. HAFED;
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Abstract: Overt and covert visual selection are guided by saliency maps of early visual features (e.g. orientation, color) and priority maps derived from cognitive factors (e.g. expectation, reward). However, the influence of higher-order visual processing (e.g. visual form recognition) on spatial maps guiding visual selection is not known. We hypothesized that if visual forms contribute to spatial maps, then they should influence saccadic eye movements even when the visual forms are irrelevant to the task. We tested this hypothesis on both humans (n = 6) and monkeys (n = 1) using a variant of the delayed visually-guided saccade task. Subjects started a trial by fixating a central spot. After 500-1000ms, two grayscale images were presented on either side of fixation at 8^{o} eccentricity. One of the images (2.5^{o} x 2.5^{o} dimensions) had a visual form (either face, body, fruit, or inanimate object), and the other image was its corresponding phase-scrambled version. After a brief delay (0.1s, 0.2s or 0.3s), the central fixation spot jumped to the center of one of the images, instructing the subjects to make an eye movement towards it. In randomly interleaved trials, only one image was presented with zero delay (simple visually-guided saccade task) in one of four diagonal locations (four quadrants) at the same eccentricity. A total of 40 images with visual forms and their corresponding phase-scrambled versions were used after normalizing for spectrum and luminance histograms. Target-directed saccades had significantly faster reaction times if the target overlapped with a visual form image compared to a scrambled image ($p<0.05$; Kolmogorov-Smirnov test). This facilitation of saccades by visual form was observed in both human and monkey subjects for all three delay periods tested. Interestingly, the effect of visual form was the strongest for anticipatory and short latency saccades. On single-target and zero-delay trials, reaction times were also faster if the underlying

image was a form rather than a phase-scrambled version. These results demonstrate that peripheral visual forms influence saccadic eye movements even when the forms are irrelevant to the task. Our findings suggest that higher-order visual processing contributes to spatial maps guiding visual selection.

Disclosures: A.R. Bogadhi: None. **Z.M. Hafed:** None.

Poster

226. Eye Movements and Perception

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Program #/Poster #: 226.10/L37

Topic: D.08. Visual Sensory-motor Processing

Support: DFG Grant EXC307

Title: Foveal action for the control of extra-foveal vision

Authors: *Z. M. HAFED[1], X. TIAN[2];
[1]Werner Reichardt Ctr. For Integrative Neurosci., Tuebingen, Germany; [2]NINDS/NIH, Bethesda, MD

Abstract: A link between tiny fixational microsaccades and covert visual attention was uncovered almost two decades ago (Hafed and Clark, 2002; Engbert and Kliegl, 2003), but it was only recently that the mechanisms behind such a link have begun to be explored. Our exploration of such a link demonstrates that neural visual sensitivity in superior colliculus (SC) and frontal eye fields (FEF) is reliably modulated at extra-foveal sites by the occurrence of "foveal" microsaccades, and this takes place even in the absence of attentional tasks (Chen et al., 2015). Moreover, such modulation is directly linked to behavioral effects (Hafed, 2013; Tian et al., 2016; 2018). Thus, there is an almost-deterministic link between the occurrence of any given microsaccade and extra-foveal visual sensitivity. However, these observations raise the question of what triggers microsaccades in attentional tasks in the first place, and why such triggering influences extra-foveal eccentricities. We ran retinal-image stabilization experiments on two rhesus monkeys to control instantaneous foveal motor error, and we investigated the effects on peripheral visual sensitivity. We also recorded SC activity in the same task from one monkey. The monkeys fixated a small 9'x9' fixation spot. For 100-500 ms, we translated the fixation spot with instantaneous eye posisition (retinal-image stabilization; every 8 ms) while forcing the fixation spot to appear 1.8' away from instantaneous gaze position (forced foveal motor error in order to bias microsaccade directions). The motor error could be in the same direction of an upcoming target, opposite it, or orthogonal to it. At the end of retinal-image stabilization, the fixation spot disappeared, while a peripheral saccade target appeared. Target eccentricity depended on recorded SC locations (typically more than 5 deg). Reaction times (RT's) on same

trials were significantly faster than all other foveal motor error directions, and there was up to a 5-fold increase in trials with express reaction times (less than 90 ms RT) compared to control trials. Neuronally, SC visual bursts after target onset were sensitized on “same” trials, similar to (Chen et al., 2015). Our results demonstrate that well-known links between microsaccades and peripheral covert visual attention likely reflect the impact of foveal motor generation on extra-foveal visual sensitivity, and not necessarily vice versa.

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Poster

226. Eye Movements and Perception

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Topic: D.08. Visual Sensory-motor Processing

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Title: Eye movements enhance visual sensitivity outside the fovea

Authors: *J. INTOY[1], N. R. BOWERS[2], J. D. VICTOR[3], M. POLETTI[4], M. RUCCI[5]; [1]Boston Univ., Boston, MA; [2]Sch. of Optometry and Vision Sci. Grad. Group, UC Berkeley, Berkeley, CA; [3]Feil Family Brain and Mind Res. Inst., Weill Cornell Med. Col., New York, NY; [4]Dept. of Neurosci., [5]Ctr. for Visual Sci., Univ. of Rochester, Rochester, NY

Abstract: Humans continually move their eyes, even during fixation, the periods between saccades in which visual information is acquired and processed. During these intervals, a physiological eye jitter known as ocular drift converts luminance patterns into temporal signals impinging onto retinal receptors. Studies focused on foveal vision have shown that humans use these temporal modulations to encode fine-scale spatial information. It remains unknown whether eye drift exerts similar effects outside the foveola. Since the receptive field size increases with eccentricity, relative changes in the spatial input caused by eye drift are expected to be smaller outside the foveola. On the other hand, it is also possible that drift-induced modulations continue to exert strong effects, as sensitivity to transients also improves with increasing eccentricity.
Here we show that drift enhances contrast sensitivity to relatively high spatial frequencies even without foveal stimulation. We measured contrast sensitivity in human observers (N=7) as they discriminated the orientation (±45^o) of 16 cpd gratings with controlled retinal image motion. Stimulation of the foveola was prevented by an artificial scotoma (1^o-diameter; stimulus at full

contrast from 1-8^{o} eccentricity) that remained stationary on the retina around the center of gaze. A real-time system for gaze-contingent display consisting of a high-resolution Dual Purkinje image eye tracker enabled precise control of retinal stimulation and accurate localization of the line of sight.
Performance was impaired when the retinal consequences of ocular drift were eliminated by stabilizing the image on the retina. Contrast sensitivity fell by 20% in the absence of luminance fluctuations from drift. To investigate the mechanisms by which drift modulates fine spatial discrimination, we controlled the amount of retinal image motion by attenuating and amplifying the impact of eye drift on the retina. We found that normal retinal image motion is optimal for performance: smaller and larger drifts reduce sensitivity. Moreover, models of retinal ganglion cells exposed to the same stimulation as experimental observers fully account for how sensitivity varies with retinal image motion. Simulated neural responses are highest for physiological drifts and decrease when drift is both enlarged and reduced. These findings show that the influence of the input reformatting resulting from eye drift is not restricted to the foveola. They suggest that the drift-induced luminance modulations are a major contributor to contrast sensitivity.

Disclosures: **J. Intoy:** None. **N.R. Bowers:** None. **J.D. Victor:** None. **M. Poletti:** None. **M. Rucci:** None.

Poster

226. Eye Movements and Perception

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 226.12/L39

Topic: D.08. Visual Sensory-motor Processing

Support: Natural Sciences and Engineering Research Council (NSERC) Discovery Grant
Natural Sciences and Engineering Research Council (NSERC) Discovery Grant Accelerator Supplement

Title: Comparing dynamic effects of expectation on motion perception and pursuit eye movements

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Abstract: Rationale: Cognitive expectations about the nature of a moving object can bias how we track and perceive that object. Expectation not only affects visually-driven smooth pursuit eye movements (Darlington et al., 2018; Deravet et al., 2018), but it also elicits eye movements in the direction of upcoming object motion, i.e., anticipatory pursuit (Santos & Kowler, 2017; Damasse et al., 2018). Expectation triggers perceptual biases that are either congruent or

incongruent with the direction or speed of anticipatory pursuit (Krauzlis & Adler, 2001; Maus et al., 2015). Here we investigate effects of expectation on the interrelation between perception and pursuit across different time scales from anticipatory to open-loop to steady-state pursuit. **Methods:** Observers (n=6 human adults) viewed random-dot kinematograms under different expectations of motion direction while their eye position was recorded (Eyelink 1000). Standard trials with 100%-coherence motion stimuli were used to build up a prior expectation of motion direction. Probability of a given direction in standard trials differed between blocks (e.g., 50%, 70%, and 90% probability of rightward motion). Interleaved perceptual trials with low-coherence motion were used to probe perceptual and pursuit biases. In each trial, participants tracked the motion with their eyes and reported perceived direction via button press. **Results:** Preliminary results reveal opposite effects of expectation on the direction of initial pursuit and perceived motion. Whereas anticipatory and open-loop pursuit were aligned with the direction of the prior (e.g., higher rightward anticipatory and open-loop pursuit velocity in blocks with higher probability of rightward motion), perceptual judgments were biased in the opposite motion direction (e.g., higher proportion of leftward judgments in blocks with higher probability of rightward motion). Interestingly, the later pursuit phase was aligned with the direction of perceptual judgments. Correspondingly, higher velocity gain was observed in the direction opposite to the prior. **Conclusions:** These results reveal a dynamic transition from following the expected motion direction in anticipatory pursuit-contrary to later perceptual reports-to tracking in line with perceptual reports during the steady-state phase. Our findings reflect a dynamic evolution of the interaction between cognitive and visual signals for pursuit control, which may differentially affect motion perception.

Disclosures: X. Wu: None. **A.C. Rothwell:** None. **A. Montagnini:** None. **M. Spering:** None.

Poster

226. Eye Movements and Perception

Location: Hall A

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Program #/Poster #: 226.13/L40

Topic: D.08. Visual Sensory-motor Processing

Support: P50-MH109429

Title: Fixation related neural dynamics during free viewing of static and dynamic images changes with the pattern of exploration

Authors: *M. LESZCZYNSKI[1,2], S. BICKEL[3], B. E. RUSS[4], M. NENTWICH[5], I. TAL[7], L. C. PARRA[6], A. D. MEHTA[8], C. E. SCHROEDER[9];
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Abstract: In natural vision, we gather information by actively scanning a scene and fixating on points of interest. Generally, humans perform 3-5 fixations/second separated by rapid "saccadic" eye movements. At each fixation, a volley of visual input is initiated in the retina and this "sample" of information is then processed by a succession of neural ensembles in areas staged along the brain's visual pathways. Previous electrophysiology and neuroimaging studies have shown that eye movements influence neural activity as early as the lateral geniculate nucleus and extending through higher order cortices including the medial temporal lobe. Little is known about whether these effects depend on dynamics of visual exploration. Using simultaneously recorded eye movements and electrocorticographic (ECoG) signals in human surgical epilepsy patients, we investigated the influence of natural visual exploration on neural dynamics. We used saccade and fixation onsets during free viewing of static and dynamic images (i.e. pictures and movies) as event points around which we could analyse eye movement-related activity. We observed a concentration of low frequency oscillatory phase activity at the frequency of visual exploration, as indexed by the inter fixation interval. This activity was strongest after but also visible before fixation onset in a distributed fronto-parietal network. In contrast broadband high-frequency activity (BHA; 70-150Hz - AKA "high gamma"), was increased in a very sparse network largely limited to parietal and occipital sites. To test whether changing dynamics of eye movement exploration influence timing, precise frequency and anatomical distribution of phase concentration we examined intervals of faster and slower saccadic exploration. We found that the frequency of strongest phase concentration followed the speed of saccadic exploration. To further investigate the role of eye movement dynamics on perisaccadic neural activity, we studied repeated free viewing exploration of the same movies. We observed hippocampal theta frequency decrease from ~6-9Hz to ~3-4Hz between first and second movie presentation, consistent with the role of theta in novelty detection and active sensing. The ongoing work aims to test whether this decrease in theta frequency might be explained by the changing saccadic exploration pattern. Altogether, our findings in these studies support an "Active Sensing" model in which the sensory input entering the brain is shaped by the motor sampling routine. Changes in the motor sampling routine (here the saccadic exploration pattern) modulate neural dynamics in anatomically distributed networks.

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Poster

226. Eye Movements and Perception

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Topic: D.08. Visual Sensory-motor Processing

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Title: Tracing the neural basis of integration of spatial information across a saccade in extrastriate visual cortex

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Abstract: Despite large disruptions in the retinal input to the visual system during a saccade, and a dramatic change between the pre- and post-saccadic visual scenes, our subjective perception of the visual world is continuous and stable. Understanding the neural basis of the integration of the pre-saccadic and post-saccadic spatial information is challenging for two main reasons: first, it is difficult to create a behavioral measure of transsaccadic integration, and second, it is experimentally challenging to measure the changes in visual sensitivity of neurons on such short timescales. To address these challenges, we develop a computational framework, along with a high spatiotemporal resolution experimental paradigm, to characterize the neuron's sensitivity across time and space at each timepoint relative to a saccade, using a set of time-varying spatiotemporal stimulus kernels. Then using a readout of the resulting high spatiotemporal resolution neuronal sensitivity map, we can examine how perisaccadic changes in the neuron's sensitivity contribute to the transsaccadic integration of spatial information. Specifically, we represent the time-varying temporal sensitivity of a neuron at each spatial location in the visual field using a set of 7 ms-wide Gaussian basis functions, defined across the time to stimulus and time to saccade dimensions for each spatial location. Using this basis function representation and an extension of the generalized linear models framework, we estimate the neuron's changing stimulus kernels, with precision on the order of milliseconds relative to the saccade time, which enables us to capture the dynamic perisaccadic response modulations. We validate our model by testing its ability to reproduce the perisaccadic response modulations of neurons in the middle temporal and V4 areas in macaque monkeys during a visually guided saccade task using a high spatiotemporal resolution visual stimulation paradigm. Using a readout of neural sensitivity across space and time provided by the model, we further examine which basis function elements are relevant to the integration of spatial information across a saccade. The resulting set of basis functions enables us to examine the role of different neural response components to the integration of spatial information across a saccade. This approach enables us to identify the perisaccadic neural correlates of transsaccadic integration.

Disclosures: **A. Akbarian:** None. **K. Niknam:** None. **K. Clark:** None. **B. Noudoost:** None. **N. Nategh:** None.

Poster

226. Eye Movements and Perception

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NRF of Korea Grant 2016M3C7A1914450
IITP of Korea Grant R7124-16-0004
ETRI of Korea, 19ZS1100

Title: Naturalistic viewing paradigm using 360° panoramic video clips and real-time field-of-view change via eye gaze

Authors: H.-C. KIM[1], S. JIN[2], S. JO[1], ***J.-H. LEE**[1];
[1]Dept. of Brain and Cognitive Engin., [2]Dept. of Computer Sci., Korea Univ., Seoul, Korea, Republic of

Abstract: We developed a novel naturalistic viewing paradigm based on real-time tracking of eye-gaze while participants were watching a 360° panoramic video during the fMRI acquisition. The gaze information of participants was recorded in an eye-tracking computer and then was transmitted to a stimulus presentation computer via the TCP/IP connection. The identified gaze position was then used to alter the participants' field-of-view (FoV) of video clip in real-time, so the participants can change their FoV to fully explore the 360° video clip (i.e., "Active" viewing). The gaze positions throughout the video watching of one participant were used to change the FoV of the same video clip for his/her matched participant (i.e., yoked, or "Passive" viewing). Four 360° panoramic videos were prepared as stimuli, in which these videos were stratified into two categories based on a brightness level (i.e., bright vs. dark) and location (i.e., nature vs. city). Each of the participants watched one of the two videos in each stratified category as "Active" viewing and the remaining video as "Passive" viewing followed by a conventional viewing with a fixed FoV (i.e., "Fixed" viewing). The data from forty-two participants out of all 48 collected were used for analysis. The representational dissimilarity matrix (RDM) codes were used in a multiple regression framework to accommodate all the information regarding the neuronal activations from fMRI and subjective ratings on viewing experience across the four video clips and two viewing conditions. Participants' naturalistic viewing experience was substantially enhanced from the Active viewing compared to the Fixed viewing and the Passive viewing. The multiple regression using the RDM codes revealed the brain regions associated with the viewing experience such as the eye movements and spatial navigation in the superior frontal area (of the Brodmann's area 6) and inferior/superior parietal areas, respectively. The brain regions potentially associated with cognitive and affective processing from the video

watching such as the default-mode networks and insular/Rolandic operculum areas were also found. To the best of our knowledge, this is the first study that used participants' eye movements to interactively change their FoV of the 360° panoramic video clips in real-time. Our method used in the MRI environment can be further extended to another environments such as electroencephalography as well as for a behavioral study. It would be feasible to apply our method to a virtual reality and/or augmented reality system to maximize a user experience using eye movements.

Disclosures: **J. Lee:** None. **H. Kim:** None. **S. Jin:** None. **S. Jo:** None.

Poster

226. Eye Movements and Perception

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 226.16/L43

Topic: D.08. Visual Sensory-motor Processing

Support: NRF-2016R1C1B2016039

Title: Spontaneous rhythmic eye movement induces active perception under ambiguity

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Abstract: When a brain receives ambiguous stimuli, a perceptual decision often spontaneously alternates between two possible states. Such perceptual switching, characterized as bistable perception, is observed to occur quasi-periodically, with frequency varying across individuals. Complex cognitive functions might be involved in this bistable perception, but in some studies (Laubrock et al., 2008; Martinez-Conde et al., 2013) it was suggested that ocular movement may solely drive periodic perceptual switches. This is because eye movement often appears as an oscillatory pattern during perceptual decision. However, whether eye movement can induce perceptual decision, or if it is merely a consequence of perception is still subject to debate. In this study, we hypothesized that oscillatory eye movement could induce an active perceptual decision for ambiguous stimuli. We performed a human psychophysics experiment with simultaneous eye tracking, using three bistable stimuli— racetrack, rotating cylinder, and Necker cube. We observed that eye gaze continuously oscillates slowly (period 1-10 s) during bistable perception. Moreover, the period of oscillation matched that of the perceptual switch in individuals. Using eye-gaze trajectory measurement only, we were able to predict when individuals would make perceptual switches. With the previous notion that switching frequency of bistable perception is strongly correlated with the sensory integration time of an individual (Choi and Paik 2019), our

results suggest that slow rhythmic ocular movement may control the temporal dynamics of sensory integration and induce active interpretation of ambiguous sensory information.

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Poster

226. Eye Movements and Perception

Location: Hall A

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Program #/Poster #: 226.17/L44

Topic: D.08. Visual Sensory-motor Processing

Support: IBS-R015-D1

Title: Prior expectation of motion direction reduces the pursuit direction variation and interneuronal correlations in macaque area MT

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Abstract: When we interact with the environment and make appropriate behavioral responses, our brain relies not only on the incoming sensory information but also on prior knowledge based on recent experience. The influence of prior knowledge becomes more prominent when the sensory evidence is weak or ambiguous. In this study, we investigated the competition between the sensory information and prior knowledge of incoming motion direction, using smooth pursuit eye movements in macaque monkeys. We controlled the monkey's prior expectation of motion direction and the strength of visual motion (100% vs 8% luminance contrast) independently. We found that the trial-by-trial variation of pursuit direction was significantly reduced when prior knowledge for motion direction was strong, especially if the sensory evidence for the motion was weak.
To understand the neural mechanisms of the effect of prior expectation on the sensory-motor behavior, we recorded responses of neurons in the middle temporal visual area (area MT), to the same visual stimulus in two different prior expectation conditions. We found that the firing rate, the direction tuning properties, and Fano factor of each neuron were not affected by prior expectation. However, the trial-by-trial correlation between activities of area MT neurons was significantly reduced by prior expectation in a 100% contrast condition. In agreement with the change in the inter-neuronal correlations, prior expectation also reduced the trial-by-trial correlation between single neural activity and pursuit direction variation in a 100% contrast condition. This result suggests that the reduction of behavioral variation by prior expectation can

be partly explained by the reduction of correlated inter-neuronal variability in population activity of area MT neurons.

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Poster

226. Eye Movements and Perception

Location: Hall A

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Topic: D.08. Visual Sensory-motor Processing

Support: Marie Sklodowska-Curie Grant 675033
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Title: Eye movement-based assessment of the perceptual consequences of ophthalmic disorders

Authors: *R. S. SOANS[1,2], A. GRILLINI[2], T. K. GANDHI[1], R. SAXENA[3], F. W. CORNELISSEN[2];
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Abstract: Assessing the presence of visual field defects (VFD) is essential in the diagnosis and management of ocular disorders. Grillini et al., (ETRA, 2018) recently proposed an alternative approach to Standard Automated Perimetry to assess the consequences of visual field defects. The goal of the present exploratory study was to evaluate this approach in a clinical context. During the test, a patient performs a visual tracking task while their eye movements (EM) are recorded. EM are intuitive to make and allow for continuous response monitoring. The test has two conditions in which the dot either moves in a continuous random walk ("smooth") or additionally moves with sudden positional displacements ("saccadic"). Subsequently, the spatio-temporal parameters of the eye movements are computed for both conditions. We assessed 13 Indian patients (10 male, age range: 17-56 yrs, mean age: 41 yrs) who were diagnosed with various ocular disorders (Primary Open Angle Glaucoma (POAG), patients who were either suspected/at risk to have glaucoma, optic atrophy, advanced glaucomatous neuropathy and hemianopia) and 5 controls (3 male, age range: 23-45 yrs, mean age: 28 yrs). The test compares a patient's performance to that of a normative control population (previously assessed in the Netherlands (age range: 30-80, 8 observers per decade, 4 male). To illustrate its outcome parameters, we highlight the case of patient P08 (male, 40 yrs) who was suspected of glaucoma. Our method confirms that while his performance on the "smooth" condition was preserved (Figs. A, C & D), his "saccadic" performance clearly deviated from the norm (Figs. B, E & F). This

result suggests peripheral vision is impaired compared to central vision, which would align with a typical glaucomatous VFD. Our results show that the spatio-temporal profiles of all remaining patients clearly deviate from the norm, whereas those of the controls did not. We conclude that the new test has clear potential as a screening tool in clinical practice and that eye movements can reveal the perceptual consequences of ophthalmic disorders.

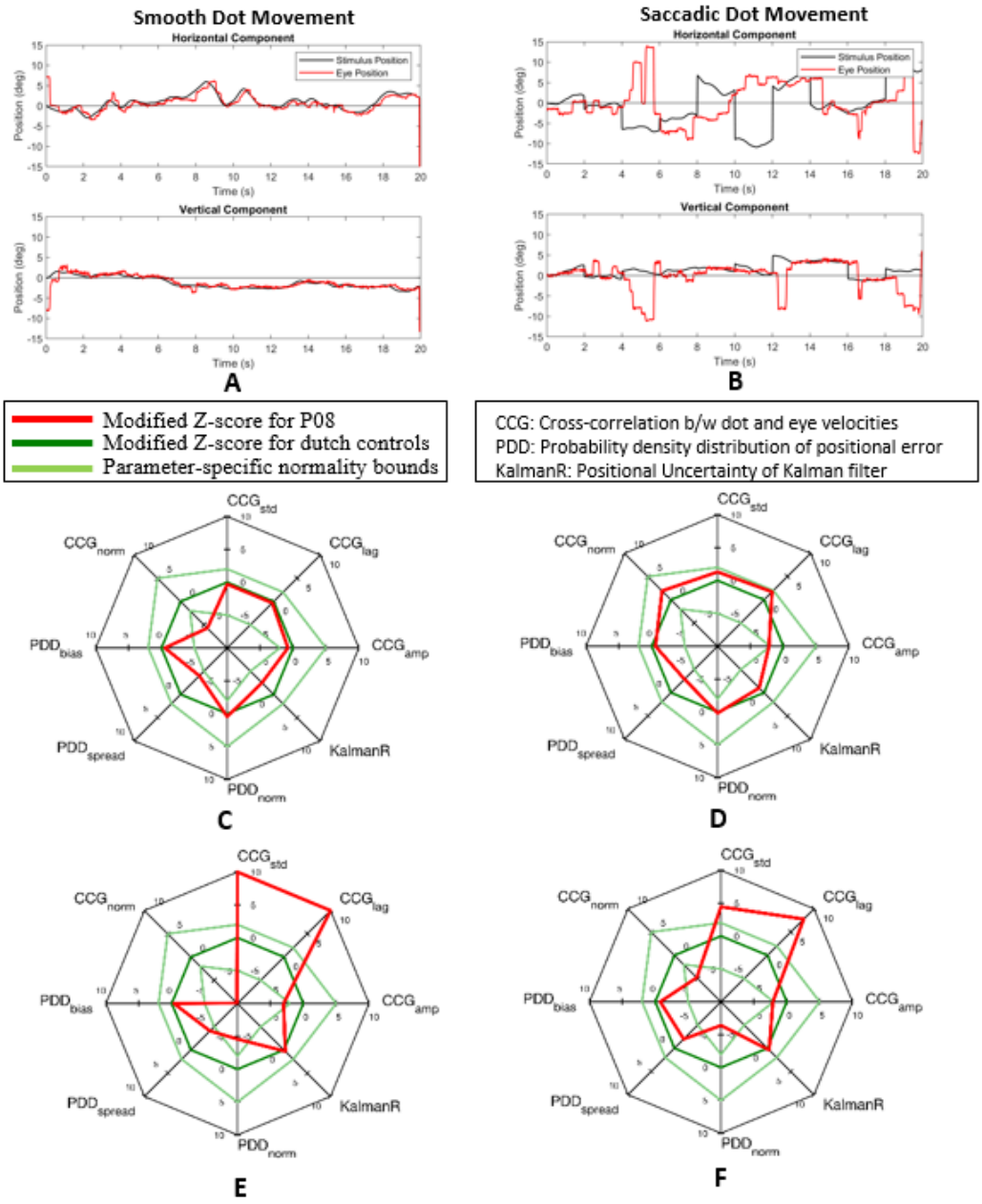

Fig. A & B: Eye movements of P08 during the two variants of the task
Fig. C & D: Spider plots of modified Z-scores for the "Smooth" parameters
Fig. E & F: Spider plots of modified Z-scores for the "Saccadic" parameters

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Poster

226. Eye Movements and Perception

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 226.19/L46

Topic: D.08. Visual Sensory-motor Processing

Title: Anti-Bayesian judgments of visual stability across saccades: Psychophysics and modeling

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Abstract: Saccades displace the visual image on the retina but external objects may also move. The visual system must decide whether displacements of images are due to eye movements or object movement. This decision is binary - did the object move? - and humans use priors about the probability of object movement for it (Rao et al. 2016). Here, we hypothesized that they are Bayesian: Subjects should rely on their prior more when the sensory evidence is noisy, as with many sensorimotor systems. In Experiment 1, human subjects (n = 20) fixated a central cross, made a saccade to a peripheral target, and made a binary judgment about whether the target had moved or not during the saccade. The probability of target movement (0.9 or 0.1) was cued by the fixation cross color. Sensory noise was varied by convolving targets with low- or high-noise Gaussian blobs. Surprisingly, subjects were seemingly Anti-Bayesian: they relied on their priors *less* when the evidence was noisy. The difference in psychometric thresholds between 0.1 and 0.9 trials (measure of prior use), in the no-, low- and high-noise conditions were $0.4^{o} \pm 0.09$, $0.32^{o} \pm 0.08$, and $0.25^{o} \pm 0.08$ respectively. To test if Bayesian behavior is lost across saccades overall and not just for binary decisions, we ran Experiment 2 where subjects (n = 14) used a mouse cursor to report the target's postsaccadic location (no binary report). As with other systems, priors influenced location estimates more for noisier targets. Thus, continuous stimulus estimates are Bayesian even though categorical judgments are not. Instead, a model of category learning in which the prior is directly stored in the weights between displacement units and binary outputs explains the data, suggesting that subjects seem to directly classify displacements as "moved" or "didn't move", a process we term "express selection," rather than implementing an optimal, generative model for Bayesian behavior. For neural studies, we are running rhesus macaques on the same, binary task. Preliminary results show that, consistent with humans, they are Anti-Bayesian. The difference in response rates between high- and low-prior trials (a measure of prior use) decreases with noise; 0.23 ± 0.04, 0.2 ± 0.05 and 0.15 ± 0.02 for no-, low- and high-noise conditions respectively. The results identify a new mechanism of prior use in the visuo-saccadic system of primates, with implications for fast decision-making processes in other perceptual categorization systems.

Disclosures: D. Subramanian: None. **M.A. Sommer:** None.

Poster

226. Eye Movements and Perception

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 226.20/M1

Topic: E.01. Eye Movements

Support: NSF GRFP DGE-1644868

Title: Predictive neural network model of perisaccadic visual responses in the oculomotor system

Authors: *A. ALERS, J. J. CHOU, M. A. SOMMER;
Biomed. Engin., Duke Univ., Durham, NC

Abstract: Around the time of a saccade, many neurons with visual receptive fields remap their visual sensitivity from their presaccadic receptive field to the region of space that will be occupied by the receptive field after the saccade. This form of visual remapping, directed parallel to the saccade, suggests that the neurons combine visual information with motor plans to predict the visual consequence of an eye movement. However, this finding of "parallel remapping" is contradicted by separate reports of perisaccadic shifts of receptive fields to the endpoint of the saccade, a phenomenon known as "convergent remapping". We have developed a model of oculomotor visual responses to study remapping. The simulated neurons are not trained to remap per se, but instead on the more fundamental goal of predicting the activity of their visual inputs. The rationale for this predictive model is that if neurons can respond more quickly by anticipating sensory inputs instead of just reacting to them, they can inform behavior that is proactive rather than reactive. Simulated neurons in our predictive model received visual and motor neural inputs defined according to neurophysiological data and were trained to predict their visual inputs in a naturalistic saccade task. Then when tested on a classical task for assessing parallel remapping, we found that the neurons replicated *in vivo* observations. Importantly, this predictive model also replicated the observations of convergent remapping in tasks used to assess it. The model appears to resolve the controversy over convergent and parallel remapping results that have been described experimentally, and it suggests hypotheses for future neurophysiological studies. Finally, the temporal properties of remapping in the model appear to provide insights into perisaccadic changes that affect the perception of time as observed in psychophysics experiments. These results support an overarching goal of prediction for visual processing in the oculomotor system.

Disclosures: A. Alers: None. **J.J. Chou:** None. **M.A. Sommer:** None.

Poster

226. Eye Movements and Perception

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 226.21/M2

Topic: E.01. Eye Movements

Title: Comparison of receptive field remapping around saccadic onset between lateral intraparietal area and frontal eye field in macaques

Authors: *L. YANG[1], C. ZHANG[1], X. WANG[1,2], N. QIAN[3], M. ZHANG[1];
[2]Sch. of Systems Sci., [1]Beijing Normal Univ., Beijing, China; [3]Columbia Univ., New York City, NY

Abstract: Our perception of the visual space remains stable despite dramatic changes of retinal images across saccades. One possible mechanism underlying this trans-saccadic visual stability is receptive-field (RF) remapping of cortical neurons around the time of saccadic onset. Specifically, some cells in lateral intraparietal (LIP) area, frontal eye fields (FEF), and other brain areas shift or expand their current, pre-saccadic RFs (cRFs) toward their future, post-saccadic RFs (fRFs), even before saccadic onset. This forward remapping have led to the Preview Theory positing that the remapping cells compare retinal images across saccades to judge trans-saccade visual stability. However, other studies show that FEF neurons shift their RFs toward the saccade target (convergent remapping) instead of toward fRFs (forward remapping) and that the convergent remapping may be responsible for attentional modulation at the target. Since different studies used different experimental and analysis protocols, it is unclear whether LIP and FEF cells have both convergent and forward shifts around the time of saccades, and whether the two areas differ in their RF dynamics. To address these questions, we recorded single units from LIP and FEF of two macaques performing a delayed saccade task, and compared RF dynamics between the two areas under matched conditions. We introduced the delay period in order to separate in time the bottom-up attentional effect from the target onset and the top-down attentional effect of the impending saccade to the target. Our preliminary data show that, during the delay period, some neurons' RFs in both LIP and FEF shifted towards the target location or a location between the target and the fixation, and a smaller number of LIP neurons had forward remapping. In contrast, during the peri-saccadic period, most remapping neurons' RFs showed various degrees of shifts toward fRF (forward remapping) and the target (convergent remapping). We also analyzed the neurons' visual response latency and found that the larger the distance between a neuron's cRF and the stimulus, the greater the response latency, consistent with our previous findings and a corollary-discharge (CD) based model for forward remapping (Wang et al., 2016). These results indicate that both attentional modulation (at the target and fixation) and oculomotor CD contribute to RF dynamics in LIP and FEF, producing various degrees of forward and convergent remapping around the time of saccades. In a

companion abstract, we describe our work on simulating both types of remapping in a single neural circuit model.

Disclosures: L. Yang: None. **C. Zhang:** None. **X. Wang:** None. **N. Qian:** None. **M. Zhang:** None.

Poster

226. Eye Movements and Perception

Location: Hall A

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Program #/Poster #: 226.22/M3

Topic: E.01. Eye Movements

Title: Properties of smooth pursuit initiation and visual motion reaction time

Authors: *S. ONO[1], K. MIURA[2], T. KIZUKA[1];
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Abstract: It has been demonstrated that the initial part of smooth pursuit is driven by visual motion related signals in cortical areas. Parietal cortex such as middle temporal (MT) and medial superior temporal (MST) areas are known to be involved in smooth pursuit initiation as well asvisual motion perception. Visual-motor reaction time (RT) has been utilized to evaluate speed of visuomotor processing which is measured as the time between the onset of the visual stimulus and the appearance of a motor response. Most studies that have dealt with visual stimulus used a light flash stimulus to evaluate motor reaction and visual perception. However, reaction time to the visual motion stimulus is an important parameter of our sensory motor processing based on visual motion perception. Therefore, the purpose of this study was to determine the relationship between smooth pursuit initiation and visual motion reaction time. We used a step-ramp paradigm to induce horizontal smooth pursuit eye movements and visual motion RT was measured to the visual motion stimuli that moved leftward or rightward. Nineteen healthy male subjects are participatedin the study. We found that some of our subjects showed directional asymmetries in initial pursuit acceleration between the leftward and rightward directions, which were consistent with an asymmetric bias in visual motion RT. Therefore, our results suggest that asymmetric pursuit initiation is associated with, at least in part, a bias of visual motion perception.These results could be due to that a common neuronal pathway in the cortical area is involved in both pursuit initiation and visual motion RT.

Disclosures: S. Ono: None. **K. Miura:** None. **T. Kizuka:** None.

Poster

226. Eye Movements and Perception

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 226.23/M4

Topic: E.01. Eye Movements

Title: Modeling circuit mechanisms of receptive field remapping in LIP and FEF in non-human primates

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Abstract: Receptive fields (RFs) of lateral intraparietal (LIP) cortex, frontal eye fields (FEF), and other brain areas show interesting remapping around the time of saccadic onset. Two main types of remapping have been reported: forward and convergent. Forward remapping refers to the shift or expansion of a cell's current, pre-saccadic RF toward its future, post-saccadic RF, even before saccadic onset, and may contribute to trans-saccadic visual stability. In contrast, convergent remapping describes the RF shift toward the saccade target, and may be related to attentional modulation of the target. Our ongoing single-unit study indicates that individual LIP and FEF neurons show various degrees of both types of remapping, under matched experimental and analysis protocols for the two areas. What circuit mechanisms, then, could account for the observed spectrum of RF dynamics in LIP and FEF? Since Wang et al (2016) already showed that corollary-discharge (CD) gated lateral connections can explain forward remapping, we first focused on possible mechanisms for convergent RF shift. It is known that in both LIP and FEF, cells tuned near and away from the attended location have enhanced and suppressed visual responses, respectively. We therefore introduced center-excitation-surround-inhibition connectivity pattern among a 2D array of model cells, in the form of a Mexican hat. When a target is just tuned on, it produces a large, transient responses for cells tuned to the target location (bottom-up attention). This then causes convergent RF shifts of nearby cells via the Mexican-hat connectivity pattern. Alternatively, if a stimulus has been on for a while but then becomes the target for the next saccade (top-down attention), convergent remapping of nearby cells can be generated by slightly modifying the connections of the cells tuned to the target location. Next, we found that Wang et al (2016)'s mechanism for forward remapping can be naturally integrated into our model by introducing a CD-gated asymmetric component to the symmetric Mexican-hat connectivity pattern. By varying the strengths of the two mechanisms, we can generate various degrees of forward and convergent remapping in model cells. We conclude that the forward and convergent types of RF dynamics around the time of saccadic onset can be realized in a single neural circuit.

Disclosures: **X. Wang:** None. **C. Zhang:** None. **L. Yang:** None. **M. Zhang:** None. **N. Qian:** None.

Poster

226. Eye Movements and Perception

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 226.24/M5

Topic: E.01. Eye Movements

Support: DFG Grant EXC307

Title: Visual-only mechanisms underlie selective peri-saccadic suppression of low spatial frequencies

Authors: ***M. BAUMANN**[1,2], S. IDREES[1,3], T. A. MUENCH[1,4], Z. M. HAFED[1,2];
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Abstract: Visual sensitivity is strongly impaired around saccades, a phenomenon known as saccadic suppression. This robust phenomenon does not constitute mere global suppression, but instead shows selectivity for low spatial frequencies, which has been used to suggest selective motor-driven suppression of magnocellular visual pathways (e.g. Burr et al., 1994). However, neural studies failed to reveal selective magnocellular pathway suppression, and in the one brain area where selective suppression was observed, superior colliculus, some neurons also showed unselective suppression instead (Chen & Hafed, 2017). Moreover, we recently found a surprisingly far-reaching contribution of visual image processing mechanisms to saccadic suppression of luminance flashes, without the need to invoke explicit motor-based suppression commands (Idrees et al. 2019). Here we show that this is also true for selective suppression of low spatial frequencies. Six participants localized a brief (~12 ms) vertical Gabor grating flashed at one of four locations (4-AFC paradigm). The gratings had one of 6 spatial frequencies (0.41-6.83 cycles/deg), and they were presented over a uniform gray background in a dark room. At a radius >10 deg from display center, the gray background was replaced by either a coarse or fine band-passed random texture (as in Idrees et al., 2019), in order to simulate a “virtual monitor” edge. In one condition, gratings were presented peri-saccadically with saccades directed towards display center; in another, gratings appeared during fixation after the “virtual monitor” and surrounding texture were translated in a saccade-like manner, again towards display center. With a coarse peripheral context, selective suppression of low spatial frequencies occurred with or without saccades, therefore due to saccade-like image translations. Even more surprisingly, when the surround was fine, both real and “simulated” saccades exhibited suppression that was not selective for spatial frequency, violating (Burr et al., 1994). Thus, selective or unselective

“saccadic” suppression happens with or without saccades, as a function of saccade-induced image translations and peripheral visual contexts. Our results support the view that saccadic suppression is a primarily visual phenomenon.

Disclosures: M. Baumann: None. **S. Idrees:** None. **T.A. Muench:** None. **Z.M. Hafed:** None.

Poster

226. Eye Movements and Perception

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 226.25/M6

Topic: E.01. Eye Movements

Support: JSPS KAKENHI JP16K09991
JSPS KAKENHI JP18K15677
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JSPS KAKENHI JP17K16271
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JSPS KAKENHI
Grant of Morinaga Houshikai

Title: Simultaneous recording of electroencephalogram and eye tracker for investigation of visuospatial attention

Authors: *M. SANEFUJI[1], Y. SHIOTSUKA[2], H. YAMASHITA[4], Y. SONODA[1], M. TORIO[1], Y. ICHIMIYA[1], Y. SAKAI[5], K. YOSHIDA[3,6], K. IRAMINA[2], S. OHGA[1];
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Abstract: [Introduction] Human visuospatial attention is often investigated using the Posner cueing task. In the task, subjects are requested to respond to attended or unattended targets that follow a cue, with fingers or eye movements. Little is known about the differential neural mechanism between the manual and gaze responses. To explore the mechanism, we conducted simultaneous recording of electroencephalogram (EEG) and eye tracker and examined the feasibility and validity. [Methods] Participants were 21 right-handed adults with normal or corrected-to-normal vision. EEG were recorded at 16 scalp sites with a sampling rate of 512 Hz using an amplifier device. Eye movements were recorded binocularly with a sampling rate of 120 Hz using an eye tracking device. A laptop computer was connected to the two devices and controlled them on MATLAB-binding software development kit. The participants performed a classical Posner task, in which they responded to the targets presented at the left or right location with mouse clicks corresponding to the side (click condition) or with eye-movements towards

the target (look condition). [Results] EEG signals and eye movements were recorded well in 13 participants. In order to correctly correlate stimulus events with eye-movement data, the clock on the eye tracker were synchronized with the clock on the computer by using Cristian's algorithm. EEG signal artifacts caused by eye movements were successfully removed by independent component analysis on EEGLAB. After these processing, we could obtain clear waveforms in both the click and look conditions. [Discussion] We successfully achieved and analyzed simultaneous recoding of both EEG and eye tracker in adult participants. This technique will reveal the differential neural mechanism of visuospatial attention between finger and gaze responses. We plan to apply this technique to children for exploring development of visuospatial attentional system.

Disclosures: M. Sanefuji: None. **Y. Shiotsuka:** None. **H. Yamashita:** None. **Y. Sonoda:** None. **M. Torio:** None. **Y. Ichimiya:** None. **Y. Sakai:** None. **K. Yoshida:** None. **K. Iramina:** None. **S. Ohga:** None.

Poster

226. Eye Movements and Perception

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 226.26/M7

Topic: E.01. Eye Movements

Title: A model of perceived location of a test-flash presented before, during and after smooth pursuit eye movement

Authors: *J. POLA, H. J. WYATT;
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Abstract: When a person makes a smooth pursuit eye movement, the perceived location of a background object does not change even though the retinal image of the object, as a consequence of the pursuit, moves from one retinal locus to another. One account of this stability of visual space is that the pursuit system generates an extra-retinal (exR) signal (e.g., a corollary discharge) resulting in a shift of perceived location relative to retinal locus, where the shift serves to cancel out what otherwise would be an apparent displacement of the object. In recent experiments designed to show the overall features of this shift (Pola & Wyatt, 2017, 2018), subjects reported on the perceived location of a 10 ms test-flash presented at various times before, during and after pursuit. These studies involved several conditions: a) subjects pursued a target that moved predictably to the right or left over trials; b) subjects pursued a target that moved unpredictably to the right or left over trials; c) exceptional subjects made pursuit-like movements without target motion. In all conditions, the shift of perceived location (determined at each time from test-flash perceived location and eye position) began shortly before pursuit, occurred more slowly than pursuit, and ended up with a magnitude less than that of the pursuit.

In short, the studies revealed how the shift occurs over time, and that the shift does not depend on the direction, predictability, or even the presence of target motion. Based on these findings, the present work offers a model of the functional properties of the neural mechanisms that underlie such a shift of perceived location. According to the model, the shift comes from an exR signal and its interaction with test-flash retinal (R) signal persistence. (Note that a 10 ms test-flash generates an R signal that persists for about 200 ms [Bowen, Pola & Matin, 1974].) The exR signal is the result of motor system dynamics (a 1st-order lag with a low frequency gain of less than 1.0 and a time-constant of about 125 ms), and the R signal persistence arises from visual system dynamics (a 5th-order lag with a time-constant of about 15 ms). With these dynamics the exR signal produces a slow shift during pursuit and a relatively low magnitude shift after pursuit, whereas the R signal persistence, interacting with the exR signal, gives rise to onset of the shift before pursuit. In sum, the model replicates the shift of perceived location found experimentally.

Disclosures: J. Pola: None. **H.J. Wyatt:** None.

Poster

226. Eye Movements and Perception

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 226.27/M8

Topic: E.01. Eye Movements

Title: Suppression of previously-fixated locations in superior colliculus during visual search

Authors: *R. NANJAPPA[1], R. M. MCPEEK[2];
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Abstract: An efficient strategy to search for a target among distractors is to explore novel regions in the scene by ignoring recently explored regions. In computational models of visual search, this is implemented by tagging recently fixated regions and suppressing activity at those locations in the priority map such that attention and saccades are biased away from these regions. To investigate the role of the primate superior colliculus (SC) during multi-saccade visual search, we recorded SC activity while monkeys searched an extended array of target and distractor stimuli. The monkeys earned reward by making a rapid sequence of saccades to fixate multiple targets among the distractors. For each cell we adjusted the search grid size and orientation so as to maximize the chance of a stimulus falling inside the receptive field of the cell with each fixation during the trial. The responses of SC neurons to a RF stimulus were often reduced when the stimulus had been previously fixated during the trial compared to when it had not. This was true both for target and distractor stimuli, and regardless of whether the subsequent saccade was directed into the RF or elsewhere. This suppression was observed in most cells 50-100ms after the onset of a fixation that brought the stimulus in the RF. Furthermore, some cells showed

anticipatory suppression of activity prior to the onset of their visual responses. Finally, we also observed suppression just before the onset of the subsequent saccade, and this suppression was much weaker if the saccade was made to the RF stimulus rather than elsewhere.
Brain areas involved in oculomotor function and attention, such as the SC, FEF and LIP, are believed to be involved in the formation of a priority map for guiding attention and eye movements. Previous studies have shown evidence for inhibitory tagging of previously-fixated items during visual search in area LIP and FEF. Here, we show that SC activity is also significantly modulated in a manner consistent with inhibitory tagging, such that SC activity is suppressed according to the history of fixation locations during visual search.

Disclosures: R. Nanjappa: None. **R.M. McPeek:** None.

Poster

226. Eye Movements and Perception

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 226.28/M9

Topic: D.08. Visual Sensory-motor Processing

Support: NIH Grant P20GM103650

Title: Characterization of natural head and eye movements driving retinal flow

Authors: *P. R. MACNEILAGE[1], C. SINNOTT[1], P. HAUSAMANN[2];
[1]Psychology, Univ. of Nevada, Reno, Reno, NV; [2]Electrical and Computer Engin., Tech. Univ. of Munich, Muenchen, Germany

Abstract: In the absence of moving objects, retinal flow is determined by eye velocity relative to the environment as well as by the structure of the environment. Eye velocity in space is the sum of head-in-space and eye-in-head velocity. To gain a better understanding of head and eye velocity driving retinal flow, we developed a system to measure both head and eye velocity during everyday behaviors outside the lab. The system consists of a Pupil Labs eye tracker with an inertial measurement unit (IMU) rigidly attached to the world camera. Head velocity is reconstructed using a computer vision algorithm known as simultaneous localization and mapping (SLAM) which works by tracking features in the scene to determine frame-to-frame image deformation, then solving for the camera motion that generated that deformation. The SLAM estimate is supplemented by angular velocity and linear acceleration data from the IMU. The result is simultaneous measurement of six-degree-of-freedom (6DOF) head velocity and binocular eye velocity. Head and eye velocity were recorded for participants walking in both indoor and outdoor environments on campus. Not surprisingly, participants tend to fixate features of the stationary environment, and robust oculomotor stabilization leads to retinal flow that is minimal near the fovea. Linear components of retinal flow are driven by linear velocity of

the head. Angular components, however, do not depend strongly on angular head velocity because angular optic flow is largely cancelled by compensatory eye movements. Instead, angular components of retinal flow are driven by compensation for linear optic flow at fixation, which depends on fixation eccentricity relative to the heading direction as well as distance to the scene. Consequently, we observe that retinal flow is driven most strongly by three factors: 1) linear head velocity, 2) fixation direction and distance, and 3) the structure of the environment.

Disclosures: P.R. MacNeilage: None. **C. Sinnott:** None. **P. Hausamann:** None.

Poster

226. Eye Movements and Perception

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 226.29/M10

Topic: D.08. Visual Sensory-motor Processing

Support: NIH Grant P20 GM103650

Title: Visual-vestibular conflict detection is modulated by motor signals

Authors: *S. J. HALOW[1], J. LIU[2], P. R. MACNEILAGE[3];
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Abstract: Head movement relative to the stationary environment gives rise to congruent vestibular and visual optic flow signals. The resulting percept of a stationary visual environment depends on mechanisms that compare visual and vestibular signals to evaluate their congruence. Here we investigate the efficiency of these mechanisms and how it depends on fixation behavior as well as on the active versus passive nature of the head movement. Sensitivity to conflict was measured by modifying the gain on visual motion relative to head movement on individual trials and asking subjects to report whether the gain was too low or too high. Low and high gains result in percepts of the environment moving with or against head movement, respectively. Fitting a psychometric function to the resulting data yields the range of gains that are compatible with perception of a stationary visual environment, referred to by Wallach as the Range of Immobility. Experiments were conducted using a head-mounted display capable of rendering visual scene motion contingent on head motion, with fixation behavior monitored by an embedded eye tracker. The experimental design included combinations of active or passive head movement together with head-fixed or scene-fixed fixation. During active conditions, subjects rotated their heads in yaw ~15 degs over ~1 sec. Each subject's movements were recorded and played back via rotating chair during the passive condition. During head-fixed and scene-fixed fixation the target moved with the head or scene, respectively. Performance was better during active than passive head movement, likely due to increased precision on the head movement

estimate arising from motor prediction and neck proprioception. Performance was also better during scene-fixed than head-fixed fixation, perhaps due to decreased velocity of retinal image motion and increased precision on the estimate of retinal image motion under these conditions. These findings quantify how visual-vestibular conflict detection is modulated by eye and neck motor signals.

Disclosures: S.J. Halow: None. **J. Liu:** None. **P.R. MacNeilage:** None.

Poster

227. Sensorimotor Coordination in Motor Control

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 227.01/M11

Topic: E.04. Voluntary Movements

Support: ERC Starting Grant 715022

Title: Is visuo-motor integration innate? Evidence from a prosthesis reaching task with individuals born without a hand

Authors: *R. O. MAIMON-MOR[1,2], A. A. FAISAL[3], T. R. MAKIN[2];
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Abstract: Despite the apparent ease with which we perform a reach, the underlying process is governed by a complex relationship between several modalities and systems. The two main inputs to this process are proprioception and vision, both providing online sensory feedback to our motor system, creating a closed control loop, known as the sensorimotor loop (Wolpert, Ghahramani, & Jordan, 1995). The integration of visual and proprioceptive inputs comprising the sensorimotor loop have been shown to be optimised based on the reliability of each sensory input (Kording & Wolpert, 2004). Here, we study whether using a prosthetic arm following limb-loss utilises similar sensorimotor hand control and feedback mechanisms. We further ask whether having had a hand is detrimental or beneficial for optimal prosthetic limb control and sensory integration. For this purpose, we tested both individuals born without a hand (congenital group, n=21), and individuals who lost their hand in adulthood (acquired group, n=16), as well as age-matched controls (n=20). Prosthesis usage was matched across the congenital and acquired groups. Participants performed a simple reaching task to a visual target with their occluded hand/prosthesis. In different conditions, hand/prosthesis reaching was performed either with or without visual feedback. When reaching without visual feedback, no differences were found between reaching errors across the three groups, indicating a ‘normal’ sense of position of the prosthesis in both one-handed groups, despite the lack of proprioceptive input from one-handers’ prosthesis. When reaching with visual feedback, the congenital group showed greater errors with

their prosthesis compared to prosthesis reaching errors in amputees, and controls non-dominant hand reaching errors. Considering congenitals' comparable performance for non-visual reaching, this finding points towards a possible deficit in integrating visual information during prosthesis motor control specifically in congenital one-handed individuals. To further confirm that reaching errors with visual feedback arise from a visual source, we calculated for each reach the distance from the target in the maximum-speed timepoint. At this early timepoint, it is thought that corrections due to visual feedback have not yet occurred, allowing us to compare performance in this condition with minimal visual-feedback influence. No group differences were found across the three groups, further strengthening our interpretation of a visuomotor integration deficit in the congenital group. Our finding reveals a potential developmental restriction of the sensorimotor loop for visuomotor integration.

Disclosures: R.O. Maimon-Mor: None. **A.A. Faisal:** None. **T.R. Makin:** None.

Poster

227. Sensorimotor Coordination in Motor Control

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 227.02/M12

Topic: E.04. Voluntary Movements

Support: JSPS KAKENHI JP16H06566

Title: Online modulation of proprioceptive reflex gain depending on uncertainty in multisensory state estimation

Authors: *S. ITO, H. GOMI;
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Abstract: To achieve precise limb movement in dynamic environment, feedback control is useful as well as feedforward control. Recent theoretical studies have suggested that online state estimation utilizing forward prediction and multisensory integration underlies the feedback control to overcome inherent delay and noise in sensory input. In addition, the feedback gain could be tuned considering reliability of estimated state to maintain optimality of the feedback control. For instance, a previous study reported a modulation of feedback gain in visuomotor control in response to changes in uncertainty of visual feedback (Izawa and Shadmehr, 2008). It is, however, still an open question if proprioceptive reflex is also modulated by the uncertainty of visual feedback through the multisensory processing of the state estimation. To clarify this point, the present study examined whether disappearance of the online visual feedback modulates stretch reflex gain. In the experiment, human participants performed wrist flexions from a start position ($\theta = 0°$) to a visual target ($\theta = 67.5°$). To manipulate uncertainty of the online state estimation, visual feedback of the hand position was removed at several timings. In a baseline

condition, visual cursor was displayed throughout the flexion movement. In the other three test conditions, the visual cursor disappeared after hand passed a certain location for each condition (Long: $\theta = 1.0°$, Middle: $\theta = 16.9°$, Short: $\theta = 33.8°$). We found significant increase in variance of end-point error in the test conditions as distance of the visual cursor removal was longer, suggesting that uncertainty of online state estimation gradually increased during the wrist movement without visual feedback. Additionally, to evaluate the stretch reflex of wrist flexor muscle, we randomly intermixed probe trials where a torque perturbation was applied when the hand reached a constant trigger position ($\theta = 50.6°$) for all conditions. We found significant decrease in amplitude of long-latency stretch reflex in condition with longer cursor removal. The results are consistent with the hypothesis that the stretch reflex gain is modulated depending on the uncertainty of the online state estimation. Our finding suggests that human motor control system employs functional tuning of feedback gain considering validity of current state which is potentially obtained through multisensory processing, even in reflexive motor control.

Disclosures: S. Ito: None. **H. Gomi:** None.

Poster

227. Sensorimotor Coordination in Motor Control

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 227.03/M13

Topic: E.04. Voluntary Movements

Title: Sensorimotor interplay in motor cortex during flexible manual interception

Authors: *Y. ZHANG, C. LI, T. WANG, H. CUI;
Lab. of Neural Mechanism of Motor Control, Inst. of Neuroscience, CAS, Shanghai City, China

Abstract: In addition to encoding various motor parameters, motor cortex has been found to carry substantial information related to sensory inputs. To elucidate the dynamic interplay between sensory inflow and motor outflow in motor cortex, we recorded population neuronal activity via a 96-channel microelectrode array (Blackrock) while macaque monkeys performed center-out flexible manual interception of a circularly moving target appearing at a random direction, and moving with five shuffled speed conditions (120° and 240°/s clockwise, 120°and 240°/s counter-clockwise, and 0°/s). For such a dynamic sensory-motor contingency, our recent results demonstrate that monkeys can fully compensate for sensorimotor delays and direct arm movements toward future target locations at interception, based on extrapolation of target motion in accordance with movement duration (Li et al. J Neurophysiol 2018). Of 39 well-isolated neurons simultaneously recorded with the Utah array, most were significantly tuned to reaching direction (36, 90%, $p<0.05$ in Kruskal-Wallis test) and/or target speed (31, 79%). Interestingly, principal component analysis (PCA) of pre-movement activity (from -500ms to movement onset) demonstrated that reaching direction could explain most variance in the first few components,

but target speed captured most variance in the last few components. Furthermore, separate PCAs of averaged movement-direction and target-speed trials showed that the first three PCs of reaching direction (target speed) trials explained 68.3% (31.7%) of the variance in movement subspace, and 17.3% (84.4%) of the variance in target subspace. Neural trajectories in each subspace were well separated in the corresponding subspace, intermingled in the other subspace. Although single neurons in the motor cortex encode both sensory and motor information in mixed and heterogeneous manners, sensory and motor variables appear to be orthogonally embodied in separate subspaces at the population level. Further analysis of population neural dynamics will help reveal the computational mechanisms underlying predictive sensorimotor control.

Disclosures: Y. Zhang: None. **C. Li:** None. **T. Wang:** None. **H. Cui:** None.

Poster

227. Sensorimotor Coordination in Motor Control

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 227.04/M14

Topic: E.04. Voluntary Movements

Support: CIHR Grant MOP125915 to LES

Title: Cognitive-motor integration performance predicts future concussion occurrence in varsity athletes

Authors: *A. E. PIERIAS[1], D. J. GORBET[2], J. HURTUBISE[4], T. MELOCHE[1], L. HYNES[1], L. E. SERGIO[3];
[2]Ctr. for Vision Res., [1]York Univ., North York, ON, Canada; [3]Sch. Kinesiol & Hlth. Sci., York Univ., Toronto, ON, Canada; [4]Dept. of Kinesiology and Applied Hlth., Univ. of Winnipeg, Winnipeg, MB, Canada

Abstract: Sport participation typically requires cognitive-motor integration (CMI), or, thinking and moving at the same time. Previous cross-sectional research from our laboratory[1,2] demonstrated CMI deficits in child, adolescent, and university-level athletes who had a history of concussion but were deemed recovered and symptom-free at the time of evaluation. Such deficits may contribute to an athlete's increased vulnerability to further injury, and partially account for the known relationship between concussion history and further concussion. In the current study, we directly tested the hypothesis that pre-season baseline CMI performance in varsity athletes was related to the likelihood of later sustaining a concussion. To this end, we recruited 103 varsity athletes (n = 35 female) from men's hockey, football, women's hockey, and women's rugby teams. Participants were tested on two visually-guided arm movement tasks. In both task conditions participants viewed targets for reaching movements on a vertical touch screen and

moved a cursor from a central target to one of four peripheral targets (up, down, left, right) by sliding their finger. In the “standard" condition, gaze and hand movements were spatially congruent. In the “non-standard” condition, eye and hand movements were made in different spatial planes and visual feedback was reversed (i.e. requiring CMI). Participants were monitored throughout their sports seasons, and then grouped into either i) having sustained a concussion following baseline testing (n = 18), or ii) not having sustained a concussion following baseline testing (n = 85). Discriminant analyses show that pre-season CMI task performance predicted whether athletes went on to sustain a concussion or not with 85.6% sensitivity. These results suggest that impaired CMI capacity may contribute to an increased vulnerability to sport-related concussion. Further, these findings support the utility of testing CMI ability as a means to provide clinically relevant information for the increased prevention of head injury.
References: 1.Brown et al.2015, BMC Sports Sci Med Rehabil.7(1):25;2. Dalecki et al.2016. Concussion, Vol. 1(3);3. Belanger et al. 2010, J Int Neuropsych Soc.16(2):262-7;4. Covassin et al.2013, Am J Sports Med. 2013 Dec;41(12):2885-9.

Disclosures: **A.E. Pierias:** None. **D.J. Gorbet:** None. **J. Hurtubise:** None. **T. Meloche:** None. **L. Hynes:** None. **L.E. Sergio:** None.

Poster

227. Sensorimotor Coordination in Motor Control

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 227.05/M15

Topic: E.04. Voluntary Movements

Support: NIH Grant R01HD059783

Title: The non-dominant arm is more accurate in unpredictable reaching conditions

Authors: *B. FOSAAEN, R. L. SAINBURG;
Kinesiology, The Pennsylvania State Univ., State College, PA

Abstract: Our lab has previously proposed a bi-hemispheric model of motor lateralization, in which the hemisphere contralateral to the dominant arm mediates predictive control processes that can achieve energetically efficient trajectories under consistent task conditions. Conversely, the non-dominant hemisphere is critical for achieving robust and stable positions under inconsistent conditions (Sainburg, 2002). While the predictions of this hypothesis have been supported for non-dominant arm movements with unexpected mechanical perturbations, we have not tested our hypothesis for reaching movements in the absence of mechanical perturbations. We designed the current study to test our hypothesis by using a task with a large number of targets (32) across the horizontal workspace. We reasoned that when first exposed to the target array, target consistency would be low (every occurrence is a new target), while with repeated

exposure to the targets, consistency should increase. Because previous research has shown that visual feedback of the moving hand facilitates predictive control processes of the dominant arm, we presented this task without concurrent visual feedback. We expect that the non-dominant arm should be advantaged for early stages of performance, when target consistency is low, while the dominant arm should be advantaged on repeated presentations of the targets. Ten right-handed young adults completed a unimanual reaching task across a 2D workspace, with the arms supported in the horizontal plane. Participants reached without visual feedback to an on-screen target with either their dominant or non-dominant arm, and were given points for accuracy when reaching within a target velocity range of between 0.5 and 2 m/s. Thus, the task emphasized both speed and accuracy. Each target appeared in one of 32 different target locations, with each participant completing 160 total reaches at the conclusion of the task (5 reaches to each target). We quantified performance by calculating final position error (FPE), the 2-D distance of the finger from the center of the target at movement completion. Remarkably, the non-dominant arm showed significantly lower errors during the early, less consistent, stage of the task ($p < 0.001$). Dominant arm accuracy became greater, after each target was repeated at least once. These findings support our hypothesis that the non-dominant arm/hemisphere system dominates under less predictable task conditions, while the dominant arm performance is greater under predictable task conditions.

Disclosures: B. Fosaaen: None. **R.L. Sainburg:** None.

Poster

227. Sensorimotor Coordination in Motor Control

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 227.06/M16

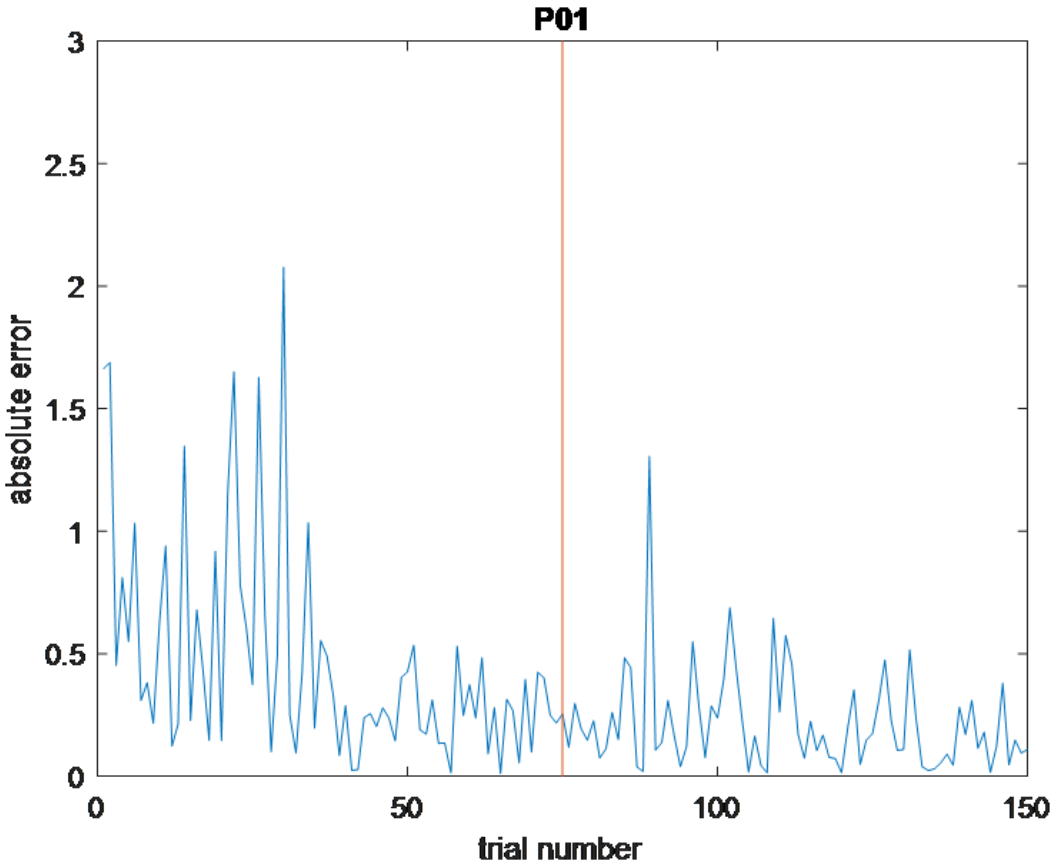
Topic: E.04. Voluntary Movements

Title: Differential effects of somatosensory and visual feedback on proprioceptive acuity and motor performance during a forearm pointing task

Authors: *Q. HUANG[1], N. ELANGOVAN[2], J. KONCZAK[3];
[1]Univ. of Minnesota Twin Cities, Minneapolis, MN; [2]Sch. of Kinesiology, [3]Human Sensorimotor Control Lab., Univ. of Minnesota, Minneapolis, MN

Abstract: Both intrinsic feedback from proprioceptive and tactile mechanoreceptors and extrinsic visual or auditory feedback play an important role in sensorimotor learning. However, the effect and interaction between intrinsic and extrinsic feedback on improving proprioceptive acuity during motor learning is only incompletely understood. The purpose of this study is to compare the effectiveness of proprioceptive, visual, and verbal feedback in improving proprioceptive acuity and while enhancing motor performance. Healthy young adult participants underwent a sensorimotor training program consisting of two sessions delivered in a single day.

Using a forearm manipulandum, participants' forearms were passively moved to a target and then back to a starting position. Subsequently, the participants actively matched the previously experienced position. After the matching movement, participants received either proprioceptive (intrinsic), visual (extrinsic), or proprioceptive/verbal (intrinsic + extrinsic) feedback about the final position error. Vision was blocked in the proprioceptive and verbal feedback condition. Each training session consisted of 75 trials. A retention test was completed 24 hours after training. Just-noticeable difference position sense thresholds (JND) as a measure of elbow proprioceptive acuity and the absolute joint position matching error (JPME) as a measure of motor performance were assessed at baseline and immediately after training. Learning occurred in all three groups. We present data on the changes in proprioceptive acuity during sensorimotor learning as a function of feedback and map how the sensory error (JND) relates to the motor error (JPME).



Disclosures: **Q. Huang:** None. **N. Elangovan:** None. **J. Konczak:** None.

Poster

227. Sensorimotor Coordination in Motor Control

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 227.07/M17

Topic: E.04. Voluntary Movements

Support: NSF Grant 1656882
NIH Grant NS058659

Title: Inability to see during the swing phase of the stride prolongs the following stance in cats

Authors: *C. T. NGUYEN[1], G. S. IYER[2], M. A. VOLGUSHEV[3], I. N. BELOOZEROVA[4]; [1]UCSD, La Jolla, CA; [2]Washington Univ., St. Louis, MO; [3]Dept Psychol, Univ. of Connecticut Dept. of Psychology, Storrs Manfld, CT; [4]Sch. of Biol. Sci., Georgia Inst. of Technol., Atlanta, GA

Abstract: Vision plays a crucial role in guiding locomotion in complex environments. However, how vision is used during locomotion is not well understood. In our recent study, we found that walking cats fixate gaze on the path in front of them while swinging each forelimb (Zubair et al., 2019). However, the swing of one forelimb is also the middle of the stance phase of the other forelimb. For humans, it was found that the end of the stance phase is indeed the time when people collect visual information about the next stepping target (Laurent and Thomson, 1988; Hollands and Marple-Horvat, 1996; Matthis et al., 2017). In this study, we examined when during the stride cats obtain visual information while walking on a complex terrain.We trained two male and one female cat to walk on crosspieces of a horizontal ladder (crosspieces 5 cm wide and spaced 25 cm apart). We extinguished lights in the room for brief periods of time (300, 400, 500, or 600 ms) during different phases of the stride. The durations of the stride's swing and stance phases were recorded with an electro-mechanical sensor placed on the paw or by an active-marker three-dimensional real-time motion capture and analysis system Visualeyez (VZ-4000, PTI Inc, Canada). We compared the duration of the swing and stance phases between strides made during continuous illumination and when lights were briefly turned off.We found that duration of a stride (a cycle from the beginning of a swing to the beginning of the next swing of the same limb) was likely to become longer if lights were off during the swing phase. The prolongation ranged between 25 and 105 ms, albeit not every stride was affected. This prolongation was due to the lengthening of the stance phase, while the swing that continued in the dark was not affected. The effect was most prominent if darkness started shortly after the beginning of a swing and most of the swing was completed in darkness. There are both similarities and differences of our findings to those reported for humans (Hollands and Marple-Horvat, 1996). Similar to humans, brief denial of vision in cats prolonged the duration of the stance rather than swing phase, and the prolongation was always shorter than the duration of the

darkness. Different from humans was the phase of the stride when cats needed to see the pathway, which was earlier in the stride. It was suggested that the biomechanics of walking shape the timing of the collection and use of visual information during walking (Matthis et al., 2015). The four-legged cat appears to exploit the greater stability of its body to look further ahead during locomotion. These findings might be relevant for developing rehabilitation approaches for patients who use a cane or walking poles.

Disclosures: C.T. Nguyen: None. **I.N. Beloozerova:** None. **M.A. Volgushev:** None. **G.S. Iyer:** None.

Poster

227. Sensorimotor Coordination in Motor Control

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 227.08/M18

Topic: E.04. Voluntary Movements

Support: Blue Cross Blue Shield Foundation of Michigan

Title: Assessing real-world arm function in children with peripheral nerve injury

Authors: *M. E. GATWARD[1], L. J.-S. YANG[3], S. H. BROWN[2];
[2]Sch. of Kinesiology, [1]Univ. of Michigan, Ann Arbor, MI; [3]Neurosurg., Michigan Med., Ann Arbor, MI

Abstract: Despite the complex nature of reaching and grasping movements in everyday activities, assessment of impaired motor function in peripheral nerve injury (PNI) is typically based on clinician-based measures of performance such as muscle strength and joint range of motion. To what extent these measures are predictive of real-world arm movement, particularly in conditions where developmental disregard may lead to significant compensatory behavior is unclear. For example, neonatal brachial plexus palsy (NBPP) is a relatively common peripheral nerve condition characterized by muscle weakness and impaired somatosensory function (Brown et al. 2016; Yang, 2014). The aim of this study was to examine the relationship between remotely-monitored arm movement in older children with NBPP and clinical measures of upper limb function. Nine children with NBPP (mean age: 11 ± 2 y) participated in the study and all attended school and participated in community activities. Standard clinical assessments included joint range of motion, muscle strength, and maximum grip force. Hand dexterity was assessed using the Nine-Hole Peg Test. Participants wore activity monitors (ActiGraph GT9X Link) that recorded acceleration in three axes (X, Y, Z) on each arm during all waking hours for 7 consecutive days. Arm movement duration and magnitude were calculated for each arm and the results expressed as the ratio of affected to unaffected arm use. These ratios were then correlated with clinic-based assessments. Significant impairments in grip force ($p=0.05$) and hand dexterity

($p<0.5$) were seen in the affected compared to the unaffected hand. Remote monitoring showed a reduction in affected arm movement in both duration and magnitude compared to the unaffected arm ($p<0.05$) but did not correlate with hand dexterity or grip force. Real-world arm movement measures were significantly correlated, however, with shoulder strength and range of motion ($p<0.01$). As we have recently shown in adults following neurosurgery to repair brachial plexus injury (Smith et al., 2018), the current findings indicate that control of shoulder motion may be the best predictor of real-world arm use in children with NBPP. Further study is required to determine the extent to which shoulder movement predicts hand dexterity in naturalistic settings in this population. Lastly, this study underscores the value of using accelerometry to augment clinical evaluation of movement deficits in peripheral nerve injury conditions.

Disclosures: M.E. Gatward: None. **L.J. Yang:** None. **S.H. Brown:** None.

Poster

227. Sensorimotor Coordination in Motor Control

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 227.09/M19

Topic: E.04. Voluntary Movements

Support: Grand-in-Aid from JSPS 26250013
Grand-in-Aid from JSPS 26120003
Grand-in-Aid from JSPS 23135533

Title: Presynaptic inhibition acts as a spatiotemporal dynamic filter of proprioceptive afferent input during voluntary movement

Authors: *S. TOMATSU[1,2], G. KIM[2], S. KUBOTA[2], K. SEKI[2];
[1]Natl. Inst. For Physiological Science, NIPS, Aichi, Japan; [2]Natl. Inst. of Neuroscience, Natl. Ctr. of Neurol. and Psychiatry, Tokyo, Japan

Abstract: Every bodily movement stimulates abundant peripheral receptors for somatic and tactile perception, and recurrently activates a numerous number of cells in the central nervous system (CNS). The central question is how the behaviorally-relevant/ irrelevant signal is selected for the regulation of ongoing behavior. We hypothesized that the reafferent sensory signals are under the control of dynamic filtering and that the presynaptic inhibition (PSI) is one of its neuronal correlates. To test this hypothesis, we applied a technique to evaluate the modulation of PSI (Wall 1958) on a muscle nerve in two macaque monkeys performing wrist flexion-extension task. We implanted an oval chamber to the lower cervical vertebrae (C4-T1) of them for intraspinal micro-stimulation (ISMS). Using a tungsten microelectrode, we applied ISMS (10 Hz, 1-50 μA) to the ventral horn and activated terminals of peripheral nerves. A nerve cuff electrode was implanted on the deep radial nerve (DR), innervating wrist extensor muscles, to

record antidromic volley (ADV) elicited by ISMS.
In total, we recorded 64 ADVs from monkeys (n = 35 and 29, respectively). Distribution of the conduction velocity of ADV was from 44.3 to 79.1 m/s (mean ± SD, 65.4 ± 8.1 m/s) suggesting the observed ADVs were induced in groups I or II muscle afferents. Next, we assessed the level of PSI by quantifying the area of ADV and tested how the input from muscle afferent is modulated relative to the onset timing of agonistic EMG activity (FDS for flexion, ED23 for extension movement). We found two types of modulation in the size of ADV. Firstly, the ADV is enhanced exclusively in the flexion trial; it started 200 ms before EMG onset and consistent throughout the task. Secondly, the ADV are reduced momentary after the EMG onset (for ~200 ms) for both flexion and extension. Since the onset of a modulation preceded the onset of EMG, it should be induced by descending motor command, at least in part. These results indicate two dominant roles of PSI on muscle afferent input; 1) a sustained suppression on the afferent input if it is coming from lengthening, antagonistic muscles (i.e., from extensor afferents during flexion movement), and 2) momentary facilitation at the onset of action (i.e., disinhibition). The former is interpreted as a sensory attenuation, the purposeful interrupt of sensory signals to highlight other information, as we reported with cutaneous afferents (Seki et al. 2003, 2009), and the latter is interpreted as an active gathering of useful information for movement control. We propose that presynaptic inhibition at the spinal level act as a spatiotemporal dynamic filter.

Disclosures: **S. Tomatsu:** None. **G. Kim:** None. **S. Kubota:** None. **K. Seki:** None.

Poster

227. Sensorimotor Coordination in Motor Control

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 227.10/M20

Topic: E.04. Voluntary Movements

Support: NSERC
BrainsCAN

Title: Spinal stretch reflexes in elbow muscles help support efficient reaching

Authors: *J. WEILER[1], P. L. GRIBBLE[3], A. PRUSZYNSKI[2];
[2]Physiol. and Pharmacol., [1]Western Univ., London, ON, Canada; [3]Brain and Mind Inst., Western University, Canada, London, ON, Canada

Abstract: It is widely assumed that the spinal circuits generating the stretch reflex in upper limb muscles only act to regulate the length of the stretched muscle. Here we show that spinal circuit also have the capability to help move the hand to a goal-location while reaching. In our first experiment participants grasped the handle of a robotic exoskeleton and reached towards a goal-location that required 10 degrees of elbow extension. At movement onset on roughly half of the

trials the robot mechanically flexed the participant's elbow - stretching the triceps - and simultaneously flexed or extended the participant's wrist. These perturbations displaced the participant's hand away from the goal-location, but critically, the perturbation that yielded the largest hand displacement relative to the goal-location did so with the least amount of elbow flexion (and vice versa). We found that the triceps' spinal stretch reflex was tuned to the hand's displacement away from the goal-location, and thus the amount of elbow extension needed to complete the reach, and not by the amount the triceps was stretched. In our second experiment participants completed a block of trials by grasping the robot handle with their thumb pointing upwards and in another block of trials with their thumb pointing downwards. Across both blocks, participants reached towards a goal-location that required 10 degrees of elbow extension and the robot mechanically flexed their elbow and simultaneously flexed or extended their wrist at movement onset. Critically, the different grasp orientations diametrically altered how the same wrist rotation moved the hand relative to the goal-location. For example, in one grasp orientation, flexing the wrist moved the hand away from the goal-location, whereas in other orientation, flexing the wrist moved the hand towards the goal-location. We found that the triceps' spinal stretch reflex was always tuned to the hand's displacement from the goal-location rather than the how much the triceps was stretched. In fact, changing the grasp orientation diametrically altered the pattern of the triceps' spinal stretch reflex and did so in a way that was appropriate for amount of elbow extension needed to complete the reach. Taken together, these results show that there is a there is a spinal circuit that integrates feedback from the elbow and wrist to support efficient reaching. These results, in conjunction with our previous findings, indicate that spinal circuits do not simply regulate the length of the muscle, but can also supports efficient hand control in a variety of contexts.

Disclosures: **J. Weiler:** None. **P.L. Gribble:** None. **A. Pruszynski:** None.

Poster

227. Sensorimotor Coordination in Motor Control

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 227.11/M21

Topic: E.04. Voluntary Movements

Support: CiHR Foundation Grant
Western BrainsCAN
Canada First Research Excellence Fund

Title: Rapid modification of an ongoing reach using touch

Authors: *S. RESCHECHTKO, A. PRUSZYNSKI;
Univ. of Western Ontario, London, ON, Canada

Abstract: Somatosensory information is available to the motor system with precision that far exceeds the capacity of psychophysical self-report, and small changes in edge orientation can be decoded early in the sensory periphery. We adapted an anti-reach task to interrogate central nervous system's ability to arbitrarily attend to subsets of stimuli on a small patch of the fingertip. Human participants made unconstrained reaches to a physical target with the dominant hand; on some trials, the target quickly moved as participants reached. A fingertip of the non-dominant hand rested on an edge whose orientation indicated the direction of target displacement. In one block of trials, participants' vision was occluded and they made reaching movements relying only on touch, while in other trials they had access to touch and vision. Critically, the axis of rotation of the edge stimulus was in the center of the fingertip such that, across the fingertip, the tactile stimulus moved in the same (distal end) or opposite (proximal end) direction as the targets. We reasoned that, if participants could attend selectively to one part of the fingertip, they would be able to make an anti-reach movement at the same latency as seen during pro-reaches toward the target. This behavior would contrast with the classical observation of visually guided reaches, in which an initial correction toward the target is observed before reaching in the correct direction. While participants were able to update their reaching strategies at the same latencies using both sensory modalities, they displayed similar latencies in both sensory conditions during the anti-reach task. However, movement kinematics and electromyograms during trials guided by touch did not show the initial correction toward the target, but rather only movement in the correct directions. These results suggest that edges are interpreted as oriented stimuli across the entirety of the fingertip but that touch-guided movements may be more easily suppressed, possibly by online modification of somatosensory gains.

Disclosures: S. Reschechtko: None. **A. Pruszynski:** None.

Poster

227. Sensorimotor Coordination in Motor Control

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 227.12/M22

Topic: E.04. Voluntary Movements

Support: The Howard Hughes Medical Institute

Title: The pontine nuclei as a link for cortico-cerebellar communication in the control of dexterous movement

Authors: J.-Z. GUO[1], ***B. SAUERBREI**[1], J. COHEN[1], M. MISCHIATI[1], F. PISANELLO[2], K. M. BRANSON[1], A. HANTMAN[1];
[1]Janelia Res. Campus, Ashburn, VA; [2]Inst. Italiano di Tecnologia, Genoa, Italy

Abstract: A growing body of work has demonstrated that the cerebral cortex and cerebellum interact bidirectionally during movement planning, Pavlovian conditioning, and cognitive function. The pontine nuclei (PN) constitute the principal brain hub that conveys signals descending from higher brain areas into the cerebellum. The PN, which consist of the basal pons and the reticulotegmental nucleus, receive a massive input from layer 5 of the entire ipsilateral cerebral cortex, and they project to both the cerebellar cortex and cerebellar nuclei. Little is known, however, about the firing properties of PN neurons during awake behavior or their feedback effects on cortical activity. Here, we use electrophysiological recordings along with optogenetic and pharmacogenetic perturbations to reveal three key aspects of PN function. First, during the execution of a cortically-dependent reach-to-grab behavior, many PN neurons respond to the movement or to non-motor events, such as the cue signaling trial start. Some neurons respond to both motor and non-motor events, suggesting that the PN do not simply relay separate channels of information into cerebellum, but perform multimodal integration. Second, motor cortical neurons that receive feedback from the ponto-cerebellar system have distinct functional properties during behavior. Third, perturbations of PN function impair performance of reaching, disrupt limb kinematics, and alter the functional responses of motor cortical neurons during movement. Taken together, these results demonstrate that the PN are a rich integrative hub required for dexterous motor control.

Disclosures: J. Guo: None. **B. Sauerbrei:** None. **J. Cohen:** None. **M. Mischiati:** None. **F. Pisanello:** None. **K.M. Branson:** None. **A. Hantman:** None.

Poster

227. Sensorimotor Coordination in Motor Control

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 227.13/M23

Topic: E.04. Voluntary Movements

Title: Gaze location changes manual size estimations of the bisecting segment of the vertical-horizontal illusion

Authors: S. YAN, T. M. SIGUR, *J. M. HONDZINSKI;
Kinesiology, Louisiana State Univ., Baton Rouge, LA

Abstract: People often perceive a longer vertical segment when viewing an inverted T (IT) configuration with segments of equal length (the vertical-horizontal (V-H) illusion). However, fixation on the V-H illusion produces stronger illusory effects on the perceived length of each segment than looking freely and may explain why illusory effects do not always exist for length estimations using hand movements. Presenting a leftward rotated T (LT, a T rotated 90° clockwise) can result in a longer perceived horizontal segment for equal segment lengths, suggesting that the bisecting segment may influence illusory perceptions. In this study, we

questioned whether people's perceptual judgments and point-to-point length estimations of the bisecting segment of the V-H illusion would vary when using different gaze strategies. After presentation of a configuration on a vertically oriented computer screen, right-handed subjects placed their dominant index fingertip on a start dot then orally reported the longer segment (vertical, horizontal) or whether they looked equal. The start dot 2 cm below an IT or right of LT represented start location. A "go" signaled subjects to manually estimate the length of the bisecting segment by lifting their finger off the screen and moving it at a comfortable pace the estimated distance in an opposite direction away from the IT (downward) or LT (rightward) before placing it back on the screen. We instructed subjects to try to ensure that the distance between the start and end of the fingertip endpoints equaled the bisecting segment length of the given configuration. Bisecting segments varied in size (49.5 mm and 40.5 mm), while bisected segments remained constant (45 mm). IT and LT presentation alternated across trials. Subjects completed the task using three gaze strategies: they gazed wherever they preferred during Free gaze; fixated on the configuration intersection during Central fixation; and tracked only fingertip motion during Finger fixation. We recorded fingertip movements using a Qualisys motion capture system and gaze directions using a SMI mobile eye tracker at 60 Hz. Subjects did not always perceive longer bisecting segment lengths; however, they manually overestimated the bisecting segment length most in Central fixation. They also manually overestimated the bisecting segment length more often for small than large configurations in Free gaze and Finger fixation. These data provide evidence that fixations directly on the V-H illusion produce greater illusory effects on point-to-point manual length estimations than when gaze is free-roaming or fixated on the movement, regardless of illusory perceptual judgments.

Disclosures: **J.M. Hondzinski:** None. **S. Yan:** None. **T.M. Sigur:** None.

Poster

227. Sensorimotor Coordination in Motor Control

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 227.14/M24

Topic: E.04. Voluntary Movements

Title: Feline head movement during locomotion over complex surfaces

Authors: ***B. D. GOPAL**[1], H. N. ZUBAIR[2], I. N. BELOOZEROVA[3];
[1]Neurobio., Barrow Neurolog. Inst., Phoenix, AZ; [2]Neurobio., Barrow Neurolog. Inst., Tempe, AZ; [3]Sch. of Biol. Sci., Georgia Inst. of Technol., Atlanta, GA

Abstract: Knowledge of how the head moves during locomotion is essential for understanding how sensory systems of the head control it. In a previous study, we investigated head movement in cats walking on a straight flat pathway in light and darkness (Zubair et al., 2016). We found that the head oscillates synchronously with strides, and this movement is largely active, with

reflexes playing only a partial role. Here we studied the head movement of cats stepping over complex surfaces (walking over barriers and along crosspieces of a horizontal ladder) in light to determine how demand on visuo-motor coordination and accuracy of stepping influences head movement on complex natural surfaces.
Three cats walked in a chamber 2.5 m long and 0.3 m wide. The chamber was either empty with a flat floor, had barriers 7 cm high, 0.5 cm thick, and spaced 6 cm apart, or had a horizontal ladder with crosspieces 5 cm wide spaced 25 cm apart. Head position and orientation was recorded with a motion capture/analysis system (Visualeyez, PTI, Canada). Head, right shoulder, and forelimb wrist LEDs were recorded at a 200 Hz sampling frequency.
We found that ladder walking speed at ~0.7 m/s was similar to that on flat surface, which is consistent with previous reports, while barriers slowed cats to ~0.35 m/s. All head left-right translations as well as yaw and roll rotations oscillated once per step cycle, while vertical translations and pitch rotations oscillated twice.
Average head height was ~22 cm during all tasks. Peak-to-peak amplitudes of vertical oscillation, however, increased on the ladder in two cats by ~0.5 cm (from 2-3 to 3.5 cm in cat 1; from 1.7-2 to 2.4 cm in cat 3; p < 0.05, t test). Vertical oscillations during stepping over barriers aligned with that of flat surfaces. Average head pitch was 17° nose down during all tasks. Pitch oscillation amplitude, however, increased on the ladder in the same two cats by 1-1.5° (from 1.8-2.6 to 3.6-3.9° in cat 1; and from 2.9 to 4° in cat 3; p < 0.05, t test). Results for the barrier task varied and in some tests were similar to those on the flat surface. During flat surface locomotion, the maximal head height occurred during the second half of each forelimb swing, following shoulder maxima by 30-85°. Both ladder locomotion and barriers overstepping increased this phase difference to 60-150°.
These results support previous conclusions that head movement during locomotion is active. They outline a limited range within which the head can move to allow successful locomotion on complex surfaces. Furthermore, they suggest that this limited range is far more consistent across different tasks than previously hypothesized.

Disclosures: **B.D. Gopal:** None. **H.N. Zubair:** None. **I.N. Beloozerova:** None.

Poster

227. Sensorimotor Coordination in Motor Control

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 227.15/M25

Topic: E.04. Voluntary Movements

Title: Pyramidal neuron types and cortical networks encoding sensorimotor activity coordinating hand-mouth synergy during eating

Authors: *H. MOHAN, X. AN, S. MUSALL, P. P. MITRA, A. K. CHURCHLAND, J. Z. HUANG;
Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Appropriate integration of sensorimotor activity is essential to coordinate dexterous movement sequences that constitute the hand to mouth feeding synergy in rodents and primates. The cortical circuit mechanisms controlling such ethological action plans requiring online sensorimotor coordination are not well understood. To bridge this gap we investigated cell type specific cortical circuit mechanisms in governing forelimb and orofacial sensorimotor features during feeding in mouse. We developed a novel head-fixed behavior paradigm closely capturing its ethological feeding sequence. Together with high speed video recording and deep learning based image recognition we tracked time course of the behavior's distinct syllables including lick, bite, hand lift, chewing and fine finger manipulation. Using optogenetic based inhibition mapping in VGAT-Chr2 mice we show that distinct areas of the sensorimotor cortex are required for the proper execution of these motor syllables. Using genetic targeting we generated cre dependent knock-in driver lines specifically capturing the corticostriatal (PyN^{PlexD1}) and corticofugal (PyN^{FezF2}) projection neurons. Expressing GCamP6f within these populations and using widefield calcium imaging we measured cell-type specific neural dynamics across dorsal cortex during the feeding behavior. Using statistical and computational modelling techniques we found distinct cortical circuits across discrete areas unique to each cell type recruited during the feeding behavior. While the PyN^{FezF2} population were specifically engaged within the frontoparietal circuits, the PyN^{PlexD1} cells were recruited within the anterolateral sensorimotor areas. Furthermore, the PyN^{FezF2} population was specifically tuned towards encoding motor components of the feeding synergy while the PyN^{PlexD1} population was strongly associated with processing sensory features. These results show that distinct sensorimotor components of the hand to mouth feeding synergy are encoded by discrete cell type specific cortical circuits.

Disclosures: H. Mohan: None. **X. An:** None. **S. Musall:** None. **P.P. Mitra:** None. **A.K. Churchland:** None. **J.Z. Huang:** None.

Poster

227. Sensorimotor Coordination in Motor Control

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 227.16/M26

Topic: E.04. Voluntary Movements

Title: Speed of hand movement does not affect proprioception during reaching

Authors: *C. R. COFFMAN, A. L. YOSS, W. G. DARLING;
Dept. of Hlth. and Human Physiol., Univ. of Iowa, Iowa City, IA

Abstract: It has been suggested that limb position and velocity are derived from the weighting of sensory inputs and efference copies of motor commands. Our recent work (Darling et al. 2018 Front Hum Neurosci 12:177), demonstrated that at self-selected movement speeds, there was no difference in accuracy of locating one's index-tip in motion whether the movement was generated by an outside entity (the experimenter) or the by the participant themselves. We extended the findings of our previous work by testing whether the target speed of passively induced movements would influence localization of the non-dominant left index-tip while moving at slow (~71 cm/s), comfortable (~93 cm/s), and fast (138 cm/s) peak speeds by reaching to it with the right dominant index-tip. We additionally tested whether active (self-generated) movement contributed to localizing the index-tip at different speeds. In 9 healthy young right-handed adults, we found no differences in locating a passively moved index-tip at different speeds ($F_{(2,16)} = 2.57$, $p= 0.11$), nor did we observe differences in variable errors between speeds ($F_{(2,16)}=0.96$, $p=0.40$) We also did not observe differences in 3D distance errors between active and passive conditions with varied movement speeds ($t_8=0.86$, $p=0.41$), nor did variable errors differ between active and passive conditions ($t_8=0.65$, $p=0.54$). Our results suggest that sensory information can be rapidly used to localize the limb endpoint, and there is no evidence that internal models contribute to kinesthesia.

Disclosures: C.R. Coffman: None. **A.L. Yoss:** None. **W.G. Darling:** None.

Poster

227. Sensorimotor Coordination in Motor Control

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Program #/Poster #: 227.17/M27

Topic: E.04. Voluntary Movements

Support: NSERC Discovery Grant 2017-04829

Title: Rapid scaling of corrective responses with movement speed

Authors: *S. V. POSCENTE[1], R. M. PETERS[1], J. G. CASHABACK[2], T. CLUFF[2];
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Abstract: Sensory feedback plays a critical role in modifying movements. Muscles are stretched when the arm is disturbed during movement. The resulting muscle stretch responses enable rapid corrections to reach a spatial target. Our previous results suggest participants may generate faster movements when interacting with environments where there is potential for the arm to be disturbed. Reaching faster requires more muscle activity, which may make the arm more sensitive to disturbances that arise during movement. Here we test this idea by manipulating the timing demands of movement. We first performed simulations based on stochastic optimal

feedback control. We controlled a virtual point mass to simulate reaching movements under three different timing conditions (300ms, 500ms, 700ms). On random trials, we disturbed the point mass with step-force perturbations. The model predicted that faster movement speeds would increase feedback gains and cause a reduction in displacement when the same perturbation was applied at the onset of movement. We conducted two experiments to test the model predictions. Twenty participants (18-35 yrs; 11 females) performed 15 cm reaching movements in a KINARM exoskeleton robot. We recorded the activity of muscles spanning the elbow and shoulder joints using surface electrodes. Our first experiment probed corrective responses by disturbing the arm with interleaved step-torque perturbations (±2Nm). The perturbations were applied the instant the participant left the start position. Participants performed reaching movements under the same timing constraints imposed on the model (within ±75ms). We found larger muscle stretch responses and a corresponding reduction in arm displacement when the same perturbation was applied during faster movements. Our second experiment assessed how feedback responses changed throughout the time course of an ongoing reaching movement. Twenty participants (10 females) performed reaching movements under two timing conditions (300±75ms, 700±75ms). Step-torque perturbations (±2Nm) were applied on interleaved trials at 0%, 33%, 67%, and 100% of the distance to the goal target. We found that muscle responses scaled with movement speed and increased when perturbations were applied closer to the end target. We observed a corresponding reduction in peak hand displacements evoked by the perturbation. Our results suggest that moving faster upregulates muscle stretch responses and reduces disturbances caused by mechanical perturbations.

Disclosures: S.V. Poscente: None. **R.M. Peters:** None. **J.G. Cashaback:** None. **T. Cluff:** None.

Poster

227. Sensorimotor Coordination in Motor Control

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 227.18/M28

Topic: E.04. Voluntary Movements

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Title: Characterizing the functional organization of spinal internal copy pathways for dexterous forelimb movement

Authors: *E. J. SANDERS[1], N. S. BALTAR[1], P. FENTON[1], H. NEDELESCU[2], P. NGUYEN[1], E. AZIM[3];
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Abstract: Dexterous forelimb movements require the coordination of dozens of muscles to propel the arm, hands and fingers through space to achieve a desired goal. The impressive precision of these behaviors is achieved in part through rapid online corrections of motor output. While sensory feedback provides information critical for assessing movement outcome, these peripheral pathways have inherent temporal delays, suggesting that rapid corrections are driven by a faster form of feedback. One proposed solution is that internal copies of motor commands are conveyed to the cerebellum, where these copies are used to predict movement outcome and make adjustments. A subset of cervical propriospinal neurons (PNs) provide an anatomical substrate for simultaneously conveying motor output and internal copies – PNs receive descending motor command input and send bifurcating axons, with one branch innervating forelimb motor neurons, and the other ascending to the lateral reticular nucleus (LRN), a major cerebellar input. Our recent work has shown that the ascending PN branch can convey signals through the cerebellum that rapidly modulate forelimb motor neuron activity. Additionally, PNs can be grouped into discrete molecular classes, suggesting a diversity of functionally distinct internal copy pathways. However, it remains unknown how these diverse copy signals are processed before reaching the cerebellum. One possibility is that separate PN classes converge onto LRN neurons, which then integrate forelimb internal copy signals before sending this information to the cerebellum, while a divergent view is that distinct copy signals remain segregated as they are conveyed to cerebellar circuits. Ultimately, establishing how cerebellar input pathways are anatomically and functionally organized will help clarify how the cerebellum uses internal copy information to correct motor output. To address these challenges, we are leveraging genetic tools in mice to: 1) Dissect the molecular diversity of neuronal subtypes in the LRN; 2) Define the anatomical organization of distinct PN inputs onto different LRN cell types; 3) Perturb LRN circuits to evaluate their contributions to dexterous forelimb movement; and 4) Characterize LRN activity during limb movements using *in-vivo* electrophysiology to define its functional organization. These approaches are revealing how a diverse group of spinal circuits convey information to the cerebellum, providing insight into how rapid internal feedback could be used to refine dexterous forelimb movement.

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Poster

227. Sensorimotor Coordination in Motor Control

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 227.19/M29

Topic: E.04. Voluntary Movements

Title: A paradigm for physiological examination of naturalistic climbing behavior in mice

Authors: A. D. SARUP, A. C. KRISTL, N. T. KOH, M. YOUNG, S. BANDYOPADHYAY, *A. MIRI;
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Abstract: Here we describe the development of a head-fixed behavioral paradigm in which mice perform a naturalistic climbing behavior. The paradigm was designed to meet several criteria that make it advantageous for a range of inquiries into motor system operation. During natural motor behavior, animals frequently negotiate unpredictable sensory stimuli. Yet behavioral paradigms used in motor system physiology have traditionally relied upon a discrete set of ballistic movements that become increasingly stereotyped over many training sessions. Recent results indicate that the execution of stereotyped ballistic movements may engage motor circuits differently than those that negotiate unpredictable sensory stimuli. Thus, our paradigm requires mice to climb along a series of handholds, the positions of which cannot be predicted from past experience. Our choice of climbing as a behavioral focus also reflects an interest in examining motor behavior more likely to have been under evolutionary pressure, for which motor system structure and function are more likely to have been adapted. Lastly, we wanted mice to perform a broad continuum of limb movements, promoting a similarly broad exploration of neural activity states. This stems from the fact that contemporary mathematical techniques useful for characterizing neural system behavior benefit from observing systems in a wide variety of activity states. However, most existing paradigms for examining limbed motor behavior in mice elicit their performance of just one or a few limb movements.
Our paradigm uses a 3D printed cylindrical wheel with radially projecting handholds. To propel wheel rotation and elicit water rewards, mice iteratively grab and pull handholds downward. Initially mounted with their hindlimbs on a horizontal platform, mice quickly transition to climbing along the handholds in an upright posture using all four limbs. After each handhold accessible to the right forepaw and hindpaw rotates past the mouse, it is rapidly repositioned by an actuator embedded within the wheel. This ensures that the sequence of right handholds mice climb along is random, so sensory information must be used in real time to steer movement. The wheel is suspended between an optical shaft encoder and a slip ring that commutes voltage signals from motor drivers to the actuators. Despite the complexity of the climbing behavior mice demonstrate, they climb naturally from their first session mounted on the wheel once they have learned the pairing between wheel rotation and reward. Thus a stable behavioral performance across sessions is achieved quickly, obviating the need for prolonged training.

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Poster

227. Sensorimotor Coordination in Motor Control

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 227.20/M30

Topic: E.04. Voluntary Movements

Support: NSERC

Title: Task-dependent responses of the eye and hand during online movement corrections

Authors: *A. J. DE BROUWER, H. M. BROWN, M. SPERING;
Univ. of British Columbia, Vancouver, BC, Canada

Abstract: To maintain accurate movement, our eyes and hand quickly correct for errors that occur during movement. Previous studies investigating error correction have often studied the eyes and hand in isolation, or in highly restricted visual environments. It is well known that the visuomotor systems performs rapid, automatic corrections in response to a displacement of a single target in an otherwise blank workspace, however, it is unclear how the eyes and hand respond to errors in richer visual environments that reflect the demands that the visuomotor system faces in the real world. To study this, we designed a set of tasks with different visual environments, in which we measured responses of the eyes and hand when making online corrections for changes in the movement goal. Human participants (n=20) performed a classic double-step task in which only one target was presented, a double-step task in which visual placeholders were presented at all three potential target positions--providing a richer visual environment, and a no-step task in which all three targets were presented and a symbolic cue indicated the reach target--additionally increasing cognitive processing demands. We found that on average both the hand and eyes initiated corrections fastest in the classic double-step task (hand: 200 ms, eye: 250 ms), slower in the placeholder task (hand: 240 ms, eye 300 ms), and slowest in the symbolic cue task (hand: 270 ms, eye: 360 ms). These findings indicate that spatial changes are processed faster in sparse environments (i.e., faster with one target than with one target and two placeholders), and are processed faster than symbolic cues. Although the hand started correcting earlier than the eyes in each task, the magnitude of the difference between hand and eye correction onset varied across tasks, with the shortest difference (50 ms) in the double-step task and the longest difference (90 ms) in the symbolic cue task. This shows that the speed of corrections is affected by the processing speed of the visuomotor system and that the relative timing of corrections of the eye and hand is adapted to task requirements. To conclude, the eyes do not lead the hand when performing rapid corrections, and although both effectors are sensitive to the number of visual stimuli and cognitive processing demands, the eyes are affected more strongly than the hand. It is possible that the visuomotor system prioritizes the response of the hand, which needs more time to complete corrections than the eyes.

Disclosures: **A.J. de Brouwer:** None. **H.M. Brown:** None. **M. Spering:** None.

Poster

227. Sensorimotor Coordination in Motor Control

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 227.21/M31

Topic: E.04. Voluntary Movements

Support: Natural Sciences and Engineering Research Council of Canada
University of Toronto Graduate Student Bursary

Title: What can audiovisual illusions teach us about gaze vs. action related spatial attention?

Authors: *T. LORIA[1], K. TANAKA[2], K. WATANABE[3], L. TREMBLAY[1];
[1]Fac. of Kinesiology and Physical Educ., Univ. of Toronto, Toronto, ON, Canada; [2]Waseda Inst. for Advanced Study, [3]Fac. of Sci. and Engin., Waseda Univ., Tokyo, Japan

Abstract: Spatial attention is influenced by both visual fixation and voluntary action mechanisms, which may subsequently affect multisensory perception. From a multisensory perspective, it is unclear if and how spatial and visual attention mechanisms interact. The current study presented the sound-induced flash illusions at movement onset, and at different positions relative to fixation (i.e., visual attention) and goal-directed action (i.e., spatial attention). Participants fixated one of three squares presented horizontally on a touch screen and pointed to the central square. At movement onset, 1-2 flash(es) were presented with 0-2 beep(s) in one of the three squares. The location of the audio-visual stimuli qualified the four experimental conditions, relative to visual fixation and the target of the aiming movement. Thus, perceptual accuracy of the number of flashes was averaged for: 1) target congruent, 2) fixation congruent, 3) target-fixation congruent, and 4) incongruent trials. Greater accuracy was associated with less susceptibility to the illusion (i.e., less multisensory perception). The results supported an additive attention-based prediction regarding the fusion illusion (i.e., induced by 2 flashes and 1 beep), such that susceptibility to fusion would be greatest when fixation and action were aligned (i.e., target-fixation congruent condition) relative to only fixating or aiming (i.e., visual or spatial attention alone). Also, the incongruent condition yielded less susceptibility to the fusion illusion than all other conditions. Moreover, there were no significant differences in fission illusion susceptibility across conditions, which can be explained by evidence that the fission illusion arises more from activity in the temporal cortex (i.e., Bolognini et al., 2011). In contrast, the fusion illusion elicits significant activity in the superior temporal cortex (i.e., Mishra et al. 2008), via projections from the posterior parietal cortex (i.e., Di Russo et al., 2003). Critically, the apparent additive effect of target- and fixation-induced modulations of the fusion illusion supports the idea of separate yet inter-related action (i.e., spatial) and attention (i.e., visual) processes on multisensory perception.

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Poster

227. Sensorimotor Coordination in Motor Control

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 227.22/M32

Topic: E.04. Voluntary Movements

Support: NSERC
University of Toronto

Title: Healthy aging delays sensorimotor transformation when planning movements to somatosensory targets

Authors: ***R. GOODMAN**, V. CRAINIC, L. TREMBLAY;
Fac. of Kinesiology and Physical Educ., Univ. of Toronto, Toronto, ON, Canada

Abstract: As humans age, their ability to plan movements to an intended target using spatial sensory information is compromised (e.g., Light & Spirduso, 1990). Specifically, while visual and somatosensory information both significantly contribute to the planning of upper-limb movements in healthy young adults (e.g., Elliott et al., 2010; Rossetti et al., 1995), it is not known how this information is utilized for movement planning in an aging population. Further, while it has been shown that older adults can exhibit movements with comparable endpoint accuracy (e.g., Helsen et al., 2016), the specific detection of visual and somatosensory stimuli has not been evaluated and contrasted in both non-movement and movement responses. The purpose of the current study was to assess the ability of older and younger adults both to identify and move to spatial targets using visual, somatosensory (i.e., tactile target), or bimodal cues. These targets were embedded in an aiming console and presented to or near the index, middle, or ring finger of the unseen left hand. Participants were instructed to lift (i.e., detection task) or reach to the target (i.e., aiming task) with their right index finger. The detection task allowed to assess the time taken to identify the presence of a target, while the aiming task was used to assess the time it took to encode the location of these targets when preparing a goal-directed movement. The results of the detection task revealed that older adults took more time to respond than younger adults, and that both groups took more time to respond to visual than somatosensory and bimodal targets. The results of the aiming task revealed that older adults exhibited longer movement onset latencies than young adults. Also, both groups took more time to initiate movements to tactile than visual and bimodal targets, and an age group by target modality interaction revealed that the temporal cost of initiating a movement to a tactile vs. a visual target was greater for older than younger adults. In sum, while older and younger adults both appeared to take less time to detect tactile than visual targets, older adults are particularly challenged when using tactile stimuli to plan upper-limb movements. Thus, the latency for older adults to utilize

somatosensory spatial information for the planning of upper-limb movements appears to be altered.

Disclosures: **R. Goodman:** None. **V. Crainic:** None. **L. Tremblay:** None.

Poster

227. Sensorimotor Coordination in Motor Control

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 227.23/M33

Topic: E.04. Voluntary Movements

Support: Trent University NSRC Grant
NSERC

Title: Assessing the usefulness of tablet-based visuomotor tasks to evaluate concussion

Authors: J. LIVERMORE[1], H. LEHMANN[1], ***L. E. BROWN**[2];
[1]Psychology, [2]Trent Univ., Peterborough, ON, Canada

Abstract: Generating and keeping track of visually guided movements is essential to many human activities. Movement performance relies on the cooperation of cortical and subcortical sensory and motor systems, and a disruption of the visuomotor system due to brain injury can result in a decreased quality of life. Evidence suggests that visuomotor system behaviour may be more sensitive to the prolonged effects of mild brain injuries than neuropsychological tests (Heitger et a. 2004; 2008). The aim of the present study was to assess the claim that visuomotor behaviour is more sensitive to assessing recovery from mild closed head injury (CHI) than standard neuropsychological tests. We evaluated whether participants with a mild CHI would show lingering visuomotor deficits, but not cognitive deficits up to three years post-injury compared to participants with a past orthopaedic injury (injury controls; IC) and healthy controls (HC). All three groups completed a tablet-based visuomotor assessment tool and a brief neuropsychological test battery. We predicted that the CHI group would perform significantly worse than the IC and HC groups on the visuomotor tasks, but perform similarly on the neuropsychological test battery. CHI participants scored comparable to the control groups on the neuropsychological tests, suggesting that they were no longer showing cognitive deficits. When assessed for visuomotor function requiring response integration or adjustment to a changing stimulus, CHI participants showed poorer performance and their performance was positively related to time-since-injury and measures of injury severity. The IC group did not show these relationships. Combined, these findings add to the evidence that CHI can lead to persistent visuomotor deficits that extend beyond those of neuropsychological tests. Visuomotor assessment should be included in brain injury and recovery evaluation and this assessment can be accomplished using tablet-based tasks.

Disclosures: **L.E. Brown:** None. **H. Lehmann:** None. **J. Livermore:** None.

Poster

227. Sensorimotor Coordination in Motor Control

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 227.24/M34

Topic: E.04. Voluntary Movements

Title: The impact of viscous and elastic loads on a bimanual reach task

Authors: *A. PABST, R. BALASUBRAMANIAM;
Cognitive & Information Sci., Univ. of California, Merced, Merced, CA

Abstract: A well-known principle of movement called Fitts's law describes a phenomenon that occurs when effectors are extended to reach targets where the target distance and size are manipulated to increase task difficulty. When reaching for two targets that differ in the length of the trajectory made, both effectors will adjust their movement trajectories so that the targets are reached at the same time. The current study investigated the impact that viscous and elastic loads would have on both movement timing and velocity of the left and right hands when load type (same load type, different load type, or no loads), strength of the load (easy, medium, and hard), and distance (left target further, right target further, or same distance) were manipulated using a bimanual KINARM exoskeleton robot. Thirty-five subjects (21 Female, 1 non-binary; aged 18 - 29yrs; 3 dominantly left-handed) were asked to reach toward the targets as quickly and as accurately as they possible could, and completed 5 trials per condition (independent variables: Distance (3 levels) X Difficulty (3 levels) X Load (4 levels) + 3 neutral conditions (no load, only varied by distance) = 39 variations, 195 trials each). Linear mixed-effect modeling revealed that load, distance, and difficulty all decreased peak velocities of both the left and right hand. Additionally, significant interactions were observed between load and distance manipulations for both left and right peak velocities: the peak velocity of the hand increased when the target on the opposite hand was placed farther away during all load conditions, demonstrating that participants were attempting to conform to Fitts's law. Surprisingly, distance was the only factor found to have a detrimental impact on the difference in reach times (slow hand movement time - fast hand movement time): any targets that had distance manipulations (left hand farther or right hand farther) had significantly increased reach differences, as we would expect to observe from Fitts's law. This suggests that while load type and difficulty may have no impact on reach differences, they do impact the velocity of the movement - suggesting that there may be different neural circuitry underlying the timing mechanisms and components of movement specific to Fitts's law.

Disclosures: **A. Pabst:** None. **R. Balasubramaniam:** None.

Poster

227. Sensorimotor Coordination in Motor Control

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Topic: E.04. Voluntary Movements

Support: NIH RO1NS111028
NIH RO1NS18338

Title: A grammar of mouse cerebral cortical activity revealed by mesoscopic Ca^{2+} imaging during natural behaviors

Authors: *L. S. POPA[1], S. WEST[1], J. ARONSON[1], R. E. CARTER[1], L. GHANBARI[2], S. B. KODANDARAMAIAH[2], T. J. EBNER[1];
[1]Neurosci. Dept., [2]Dept. of Mechanical Engin., Univ. of Minnesota, Minneapolis, MN

Abstract: Understanding both brain function and pathology has to include characterization of the rules governing the neural network at a global level. Chronic implants of morphologically conformant, optically clear, skull prostheses allow access to large areas of the dorsal cerebral cortex (~49 mm^2) in Thy1-GCaMP6f mice, expressing a Ca^{2+} indicator in excitatory neurons. This method allows long-term mesoscopic imaging of cerebral cortical activity in behaving animals head-fixed on a free-moving disk. Mesoscopic imaging was performed at 20 Hz in 5 min trials. High-definition video cameras simultaneously recorded behavior that was classified off-line using machine learning algorithms. Data was separated in 30 sec epochs of either awake quiescence or locomotion. We functionally segmented the imaged cerebral cortical area into independent components (ICs) during each epoch. Each IC included one or more contiguous domains. Based on two dimensional correlations, we generated a catalog of 36 domains that exhaustively covered the imaged cortex. We obtained 288 different ICs based on these domains. We also observed that a large majority (32/36) of individual domains can multiplex, that is can be part of more than one IC in the same epoch. Based on this classification we characterized the increased complexity of cerebral cortical activity during locomotion epochs. Averaged number of domains in an IC increased from 1.6 ± 0.24 during quiescence to 2.0 ± 0.25 domains during locomotion (two sample t-test, $p < 0.001$). Also, the average number of ICs increased from 7.4 ± 2.4 during quiescence to 13.0 ± 1.7 during locomotion (two sample t-test, $p < 0.001$). The average frequency at which domains engaged in multiplexing also increased during locomotion from 0.05 ± 0.07 to 0.19 ± 0.25 (two sample t-test, $p = 0.003$). Graph analysis of domain contingency frequency shows a large majority of the cataloged domains (33/36) have stable nodal degrees irrespective of the behavioral state. Only three domains, located in or close to the visual cortex, were specific to locomotion. In contrast, the ICs are remarkably segregated, with 102 ICs specific to quiescence and 121 ICs specific to locomotion. These preliminary results

suggest that the cortical state can be described by a grammar consisting of 36 “letters” (i.e., the domains) that generates a large dictionary of “words” (i.e. the ICs). In turn, following behavior related “syntactic rules”, subsets of words describe the cerebral activity associated with behavioral states.

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Poster

227. Sensorimotor Coordination in Motor Control

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Program #/Poster #: 227.26/M36

Topic: E.04. Voluntary Movements

Support: NIH R01 NS111028
NIH R01 NS18338
NIH T32 MH115688

Title: Wide field-of-view calcium imaging of dynamic cortical networks underlying locomotion

Authors: *S. L. WEST[1], J. D. ARONSON[1], L. S. POPA[1], A. C. SHEKHAR[1], R. E. CARTER[1], L. GHANBARI[2], S. B. KODANDARAMAIAH[2], T. J. EBNER[1];
[1]Dept. of Neurosci., [2]Dept. of Mechanical Engin., Univ. of Minnesota, Minneapolis, MN

Abstract: Locomotion—the act of moving from one location to another—involves many cerebral cortical areas. It is unclear how these different cortical regions interact during locomotion to produce a coordinated behavioral output, how these interactions differ from those at rest, or how these regions are recruited into the locomotive functional state. Optically clear, morphologically conformant polymer windows are implanted in the skulls of Thy1-GCaMP6f mice expressing the Ca^{2+} indicator GCaMP6f in Layer II/III and Layer V pyramidal neurons. These prosthetic windows allow for wide field-of-view (approximately 7X7 mm) for optical imaging of the majority of the dorsal cerebral cortex at 1x magnification. The mice are head fixed on a freely moving disk that allows free limb and body movements including waking. Wide field-of-view Ca^{2+} imaging is performed during rest, spontaneous locomotion, and cued locomotion (20 Hz acquisition at a spatial resolution of 25 x 25 μm). Behavior is recorded with infrared cameras at 20 Hz simultaneously with brain imaging. Paw movements are tracked using the deep learning algorithm, DeepLabCut, and used to classify behavior as rest, locomotion, or other behaviors such as grooming. Animals are recorded across multiple sessions, each with 10-15 recordings lasting 5 minutes each. The wide-field imaging data is divided into independent functional regions using spatial independent component analysis (ICA). Time series are extracted from the ICs and correlated to compute functional interactions, which reveal dynamic functional

networks in the mouse cortex in relation to locomotion. Preliminary analyses show that just before the animal begins walking, there is an overall increase in correlation across cortical region. This increase subsides as locomotion proceeds. The functional cortical network then changes in shape, with individual functional regions becoming associated with different sub-networks during locomotion than at rest. Understanding these cortical interactions in rest and locomotion will help elucidate how the cerebral cortex generates the locomotor state.

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Poster

227. Sensorimotor Coordination in Motor Control

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Program #/Poster #: 227.27/M37

Topic: E.04. Voluntary Movements

Support: NIH Grant 5K99NS107721-02

Title: An amygdala circuit for experience-dependent exploratory arrest

Authors: *P. BOTTA[1], A. FUSHIKI[1], A. VICENTE[1], A. C. MOSBERGER[2], R. M. COSTA[3]; [2]JLG Sci. Center, L3-003, [3]Neurosci., [1]Columbia Univ., New York, NY

Abstract: In a world varying in time and space, exploration of new environments ensures survival and evolutionary fitness of species. Using an unrestrained exploratory behavioral assay, we found an experience-dependent emergence of voluntary arrests in defined familiar and preferred locations. While it is suggested that these arrest areas are crucial in shaping the entire exploratory repertoire of a variety of species, the neuronal basis of their dynamic formation remain elusive. The combination of temporally precise speed-dependent closed-loop optogenetic manipulations and quantitative analyses of neuronal calcium activity in freely-exploring mice revealed that a large basolateral amygdala neural ensemble encodes self-paced behavioral arrests. While arrests-encoding amygdala neurons are recruited in an experience-dependent manner, location-specific inhibition strongly delays the long-term formation of exploratory arrests. Neurons of basolateral projecting to central amygdala cause arrests without inducing long-term effects. These findings uncover for the first time the experience-dependent formation of a new amygdala neuronal ensemble recruited for location-specific voluntary arrests during exploration.

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Poster

228. Motor Control in Primates and Humans

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Program #/Poster #: 228.01/M38

Topic: E.04. Voluntary Movements

Support: CIHR (MOP-102662)
CFI
FRSQ
FRQNT

Title: Dynamics of the neural state in premotor and parietal cortex during multi-attribute decision-making

Authors: *A. NAKAHASHI[1], P. E. CISEK[2];
[1]Neurosci., Univ. of Montreal, Montréal, QC, Canada; [2]Univ. of Montreal, Montreal, QC, Canada

Abstract: During a delay period, activity in sensorimotor regions reflects not only the action to be executed, but also the decision variables pertinent to the ongoing task. Using a value-based, multi-attribute decision task, we previously reported that the activity of neurons in monkey dorsal premotor cortex (PMd) predicted choices faster than posterior parietal cortex (PPC), and that conflict resolution seemed primarily driven by biases in baseline activity in PMd, but not PPC. While these results suggest a causal role of PMd in the decision process, it is unclear if PPC is also involved. Here, we analyze the activity of the entire population by plotting the dynamical state of the system as a trajectory through a high-dimensional neural space based on the activity of each neuron. We then use Principal Components Analysis to reduce this into a lower-dimensional projection and examine how neural trajectories of PMd and PPC evolve over time in different conditions. As in previous analyses of activity in a very different decision task, we found that the three strongest components reflect the transition from deciding-to-acting, the upcoming choice, and a time-varying signal possibly related to the rising urgency to act. In addition, we found that during deliberation, the neural state of PMd lies on a convex “decision manifold”, similar to that previously observed during a temporally dynamic decision task, and possibly indicating a normalization of neural activity across the PMd population. In contrast, the decision manifold in PPC was planar, similar to our previous analyses of primary motor cortex. Finally, the difference in trial types was reflected in the time course of activity, most prominently in the choice-related component. The fastest choices appeared when only one target was displayed, i.e. when no decision was needed. This was followed by a group of trials in which one target was clearly better than the other. The slowest choices were seen in trials presenting the monkey with a conflict, with timing dependent on how the monkey resolved that conflict. The

order of these effects was observed in neural state components in both PMd and PPC, though at the single-cell level it was only visible in PMd.

Disclosures: A. Nakahashi: None. **P.E. Cisek:** None.

Poster

228. Motor Control in Primates and Humans

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Program #/Poster #: 228.02/M39

Topic: E.04. Voluntary Movements

Support: JST JPMJPR18J6
NTT Network Innovation Laboratories

Title: Successive failures caused by exploratory action in a reinforcement-based motor task

Authors: R. SAITO[1], M. KODAMA[2], D. NOZAKI[1], *M. TAKEMI[1,3];
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Abstract: Motor variability is not only deemed as an unwanted byproduct of a noisy nervous system (Churchland et al. 2006) but also crucial for reinforcement learning as it allows the nervous system to find new task solutions through exploration (Wu et al. 2014). Here, a key question is how motor variability changes trial-to-trial in a reinforcement-based motor task. We hypothesized that motor variability increases immediately after an unrewarded failure trial triggering exploratory action (Pekny et al. 2015) and decreases with the number of trials by reinforcement learning. To this end, we designed a reaching task with only reward-based feedback (binary success/failure) and quantified whether the performance at a trial modulates reach variability in the subsequent trials.
Seven healthy adults participated in the experiment. In each trial, a visual target was shown 10 cm away from a starting position. Participants reached and attempted to pass through the target without visual feedback of hand position. Success/failure was defined by whether the hand path overlapped with the target. The target size, which would result in a success rate of 50%, was adjusted individually using baseline reach variability and kept constant for 20 min of the experiment.
Contrary to our hypothesis, the results did not show an increase in motor variability quantified by the minimum distance from the center of the target to the hand path in the trials immediately after failure trials. However, in all participants the probability of occurrence of two successive failures was higher than the square of the failure probability in the whole experiment, suggesting that a failure streak occurred above the chance level. To explain these results, we developed a model in which motor variability is decreased in each trial by learning and increased after failure

trials by exploration. This model was better to predict a behavior of successive failures in a reinforcement-based motor task than the models incorporating either learning or exploration factor. Next, we assigned thirty-eight adults to the conditions where the target size adaptively changes according to their motor performance. The results showed that in the thirty-four participants the probability of successive failures was higher than the chance level and was better predicted by the model with both learning and exploration factors. In summary, we found that exploratory action induced by failed performance may cause successive failures in motor tasks, thereby possibly being a mechanism underlying a failure streak, so-called motor slump, in athletes.

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Poster

228. Motor Control in Primates and Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 228.03/M40

Topic: E.04. Voluntary Movements

Title: Task complexity can influence kinematics of goal-directed arm reaching movements in chronic stroke survivors - A pilot study

Authors: *B. KIM, G. MAMMOLITO, T. AGAG, J. PABULAYAN, J. GIRNIS, V. WALLS, T. NOBILING;
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Abstract: Background: Stroke survivors develop compensatory movement strategies to substitute their motor impairment. It is not well studied how task conditions influence compensatory movement strategies in chronic stroke survivors. This study aims to determine how task difficulty and complexity affect the kinematics of goal-directed arm reaches in chronic stroke survivors. Methods: We recruited two non-disabled young adults and two chronic stroke survivors with mild upper extremity motor impairment. Each participant performed goal-directed arm reaching tasks with the following conditions: 1) pointing large target (2 X 2 cm^2) with a stylus; 2) pointing small target (0.3 X 0.3 cm^2) with a stylus; 3) picking up a large object (2 X 2 X 2 cm^3 wooden cube) with a pair of chopsticks; 4) picking up a small object (0.3 X 0.3 X 0.3 cm^3 plastic cube) with a pair of chopsticks. Non-disabled participants performed the tasks with their dominant arm, and chronic stroke survivors performed with more affected arm, which was a dominant arm before the stroke. Participants held a stylus or a pair of chopsticks and placed the

tip of the stylus or chopsticks at home position. They started reaching their arm toward the target located 20 cm in front of the home position when they felt the sensory-level electrical stimulation on their non-dominant or less-affected arm provided as a cue to initiate arm reaches. They repeated 10 reaches per task condition. Positions of the trunk, upper extremity, and the tip of chopstick were recorded using a motion capture system. Kinematic variables of goal-directed arm reaches were calculated. We used shoulder marker trajectory length as a measure of trunk compensation. Results: In both non-disabled adults and chronic stroke survivors, there was a significant increase in movement duration, movement jerkiness, and trunk compensation as a function of task complexity, but there was no difference in these variables between different task difficulties. Further, chronic stroke survivors showed significantly greater movement duration, dimensionless jerk, trunk compensation, and less peak velocity than non-disabled young adults. Discussion: Our preliminary results support that task conditions can influence motor control strategies in chronic stroke survivors even they are mildly impaired. Specifically, chronic stroke survivors used slower and less smooth movements and more trunk compensation for a more complex task that requires a certain level of hand dexterity. A better understanding of the impact of task conditions on the compensatory movements can help us to optimize therapeutic interventions for upper extremity motor function in individuals after stroke.

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Poster

228. Motor Control in Primates and Humans

Location: Hall A

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Program #/Poster #: 228.04/M41

Topic: E.04. Voluntary Movements

Title: Go/No-go decision making under severe time constraints interferes with hitting task performance

Authors: *A. KOBAYASHI, T. KIMURA;
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Abstract: Fast and accurate decisions and motor adjustments are required in many sports. In baseball, for example, the flight time of the pitched ball (time-to-contact: TTC) is frequently less than 0.5 seconds. Batters must distinguish strike balls and hit them accurately even though the time constraints are severe. We posit that when the time constraints are very severe, decision making may interfere with motor execution because they are assumed to be serial rather than parallel processes. For very fast pitches, batters sometime take the heuristic strategy of attempting to hit all balls without deciding the ball's likely status (strike or no-strike). To confirm our assumption, we examined the effect of consciously judging ball status on cognitive

and motor performance in a manipulandum-based experiment (KINARM, BKIN Technologies, Canada) with different TTC values (0,4, 0.5 and 0.6s). Participants were instructed to attempt to hit the moving target in the desired (strike) zone by manipulating their hand cursor from the start position so as to hit the target and send it back into the goal zone. The G-strategy (Str-G) required the participants to attempt to hit the target only if its trajectory was judged to pass through the strike zone (Go, 66%), otherwise not to swing (No-go, 33%). The A-strategy (Str-A) required the participants to swing at all balls regardless of the perceived trajectory. This strategy yielded a higher goal rate for strike balls than other balls regardless of TTC, indicating that participants should hit strike balls and exclude other balls in order to improve the goal rate. As expected, for TTC values of 0.5 and 0.6, Str-G yielded higher hit rates than Str-A. Both strategies yielded the same spatial error of hand movement, which was defined as the contact angle between the cursor and the ball for strike balls. At the TTC value of 0.4 s, however, Str-G yielded larger spatial error than that of Str-A. In separate sessions, participants were asked to press a button immediately upon judging if the ball was a strike. It was found that there were significant differences in the d' value as calculated by signal detection theory, for 0.4 TTC, but not for 0.5 and 0.6 TTC. This behavior is identical to the hit rate and success pass rate in the hitting experiment. In addition, at 0.4 TTC, participants who had slow reaction times for strike judgment tended to show large spatial error. These results suggest that, 1) cognitive judgment and motor execution are linked in a serial process and, 2) Go/No-go decision making interferes with both cognitive and motor process under very short time constraints, (TTC of less than 0.5 seconds).

Disclosures: **A. Kobayashi:** A. Employment/Salary (full or part-time):; NTT Communication Science Laboratories. **T. Kimura:** A. Employment/Salary (full or part-time):; NTT Communication Science Laboratories.

Poster

228. Motor Control in Primates and Humans

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Program #/Poster #: 228.05/M42

Topic: E.04. Voluntary Movements

Support: Sao Paulo Research Foundation (#17/26147-9)
American Society of Biomechanics Grant-In-Aid

Title: Wrist orthoses lead to kinematic changes associated with an increased risk of shoulder dysfunction

Authors: H. T. TUCCI[1,2,5], ***E. M. BAILLARGEON**[2,3,5], D. LUDVIG[2,5], A. L. SEITZ[3], E. J. PERREAULT[2,4,5];
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Abstract: Commercial orthoses are often used to treat wrist neuromusculoskeletal diseases. While effective for treating the wrist, limiting wrist motion may also cause compensatory changes proximally, as accurate reaching requires the coordinated action of all arm joints. The shoulder is of particular importance as it is responsible for positioning the hand in space. In addition, compensatory changes in shoulder glenohumeral (GH) kinematics, specifically elevation and internal rotation, have been linked to shoulder dysfunction and injury risk. Currently, it is unknown how wearing a wrist orthosis will influence GH motion. Therefore, the objective of this study was to determine how wearing a commercial wrist orthosis alters GH kinematics during reaching-to-grasp tasks. We hypothesized that there would be an increase in GH elevation and internal rotation to compensate for the restriction in wrist motion.
Nine right-handed adults (6 female, 3 male, 30±6 years; mean ± standard deviation) performed reach-to-grasp tasks while we measured motion of the right upper extremity using active surface markers. We chose tasks that varied upper extremity kinematics and grasp type to explore the influence of an orthosis across a range of activities. Participants performed 5 repetitions of 8 tasks at a self-selected pace, then repeated the protocol wearing a commercial wrist orthosis. We computed 3D shoulder kinematics and calculated the mean and range of GH (humerus relative to scapula) elevation and internal rotation during each task. We then used linear mixed-effects models to compare the mean and range of each angle during trials with and without the orthosis. When wearing the wrist orthosis, participants maintained a more elevated GH position and had a smaller elevation range of motion during reach-to-grasp tasks. The mean GH elevation was 7.0±0.5 degrees (mean ± standard error) higher ($p<0.001$, $t=15.3$) and the range of GH elevation was 8.2±0.6 degrees smaller ($p<0.001$, $t=13.2$) with the orthosis on than with the orthosis off. In contrast, we found no significant difference in the mean ($p=0.88$, $t=-0.2$) or range ($p=0.73$, $t=0.3$) of GH internal rotation across all tasks with and without the orthosis. These results demonstrate that wearing a wrist orthosis leads to some of the compensatory changes at the shoulder commonly associated with dysfunction.
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Poster

228. Motor Control in Primates and Humans

Location: Hall A

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Dynamic Poster
Topic: E.04. Voluntary Movements

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Title: Manipulating complex objects in the face of perturbations: Predictability through stability

Authors: *S. BAZZI[1], D. STERNAD[2];
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Abstract: Manipulation of complex objects is ubiquitous in our daily life, yet to date this question has been only little explored in motor neuroscience. Consider the task of guiding a cup of coffee to one's mouth: this complex interaction between the hand and the cup filled with liquid gives rise to bidirectional forces which need to be predicted and compensated for. However, due to the slow neural transmission and intrinsic noise, compensation through feedback control in such complex interactions is insufficient. Moreover, prediction based on internal models of these dynamically-complex objects seems implausible. We hypothesize that humans seek to make these interactions predictable by exploiting stability. A stable system obviates the need for error correction and returns to its orbit when perturbed. In previous work we have shown that when moving a complex object over visible perturbations in a discrete movement, subjects stabilized their trajectories within the vicinity of the perturbation to attenuate its perturbing effect. Stability of the trajectory was evaluated using contraction analysis that identified contracting subspaces in state space. Results of four subjects showed that they indeed navigate the perturbation by seeking stability. State space analysis showed that their trajectories visited the convergent ("stable") subspace with more practice. However, the options for the subjects to explore the state space were limited as they always started with the same initial conditions. This study tested the same hypothesis and allowed subjects to choose the initial state of the cup and ball before starting their movements to allow for richer behavior and robust control strategies that successfully complete the task. Using a virtual set-up, a simple cart-and-pendulum model mimicked the task of transporting a cup of coffee: the pendulum bob represented the liquid moving inside a cup defined by the bob's semicircular path. Participants moved a robotic manipulandum to control the virtual cup with the ball "rolling" inside; the goal was to move the cup to a target as fast as possible without losing the ball. A small perturbation assisting or resisting the motion was presented at a fixed location along the path. At the beginning of each trial, subjects were instructed to move the cup-and-ball system back and forth freely and once the system reached the initial state that they desired they could then move towards the target. Contraction analysis was used to assess the stability and robustness of the control strategies employed by the subjects. Preliminary results show that again subjects direct their trajectories to regions in state space that provide stability.

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Poster

228. Motor Control in Primates and Humans

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Title: Dynamic primitives account for human constrained motion

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Abstract: Despite slow muscles and long feedback delays, humans excel at physical interaction with complex objects. To study strategies underlying physical interaction, we examined kinematically-constrained circular arm motion (crank-turning) as an intermediate step to bridge the gap between (widely-studied) unconstrained motions and (sparsely-studied) physical interaction with complex dynamic objects. Imperfect execution of planned motor actions may be anticipated due to sensor noise, motor noise, or inadequate compensation for inertial and neuromuscular dynamics. In the crank-turning task, the consequences of these imperfections should be independent of turning direction (clockwise vs. counter-clockwise). However, the strategy used to perform the task may introduce differences. In particular, if subjects take advantage of neuromuscular mechanical impedance to reduce required control precision, this may introduce a lag between nominal and actual motion that differs with direction. Hypothesis: The underlying neural commands will exhibit differences between directions. Ten right-handed male subjects turned a crank (radius 10 cm) in two directions at three constant instructed speeds (fast, medium, very slow) with visual speed feedback, completing 23 trials at each speed. To disentangle the influences of biomechanics and neural control we assumed a plausible mathematical model of interactive dynamics and used it to 'subtract' peripheral biomechanics, revealing underlying neural influences expressed in terms of motion. We called this data-driven construct the zero-force trajectory. The observed zero-force trajectory was approximately elliptical. The principal eigenvector of its covariance matrix served to estimate the orientation of the ellipse major axis. While turning speed did not affect the orientation, turning direction had a significant effect. Constant-speed circular hand motion consists of two orthogonal sinusoids at the same frequency. Peripheral neuromuscular compliance (i.e. low mechanical impedance) mitigates the

consequences of imperfect execution, reducing the required precision of motion commands. To produce circular hand motions, this control strategy requires an oscillatory zero-force trajectory that leads hand motion. Due to non-isotropic dynamics, that lead differs between degrees of freedom. The result is an elliptical zero-force trajectory with orientation that differs with direction of rotation, just as we observed. These results indicate that in this constrained-motion task humans use dynamic primitives, oscillations and impedance, and do not have to compensate for limb and object dynamics.

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Poster

228. Motor Control in Primates and Humans

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Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

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Topic: E.04. Voluntary Movements

Title: Conceptual similarities between targets and distractors influence visually-guided reaching

Authors: *C. B. MARTIN, Z. CHENG, J. PANG, M. D. BARENSE;
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Abstract: The ability to adaptively interact with a complex and cluttered world requires that we concurrently process the meaning and visual form of multiple objects. Previous research has demonstrated that competing action plans are simultaneously represented in the brain when there is uncertainty regarding which object among an array will ultimately become a target. Behaviourally, this competition can be quantified as the bias toward a distractor in continuous measures of rapid reach movements. In the current study, we examined how visual and conceptual similarities between objects influenced visuomotor decisions in the context of a rapid reaching task. Importantly, degree of visual and conceptual relatedness varied independently across potential targets. Reach trajectory was indeed influenced by conceptual similarities between targets and distractors. Critically, when participants reached to a target in an automatic manner (i.e., rapidly initiated movement), bias toward distractors increased linearly with degree of conceptual similarity. Conversely, when participants reached to a target in a controlled manner (i.e., slowly initiated movement), bias toward distractors decreased with increases in conceptual similarity. In other words, automatic movements deviated more toward conceptually similar than dissimilar distractors, whereas controlled movements deviated more toward conceptually dissimilar than similar distractors. Reach trajectory was not influenced by visual similarity when words were used as stimuli. These results suggest that the meaning of objects critically shapes competition among multiple action plans prior to the decision to act on a target. Moreover, they highlight an important interaction between conceptual similarity and automatic versus controlled movement.

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Poster

228. Motor Control in Primates and Humans

Location: Hall A

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Program #/Poster #: 228.09/N2

Topic: E.04. Voluntary Movements

Support: NSERC

Title: Is visual search for target objects that will be acted upon influenced by motor costs?

Authors: *J. B. MOSKOWITZ, S. A. BERGER, M. S. CASTELHANO, J. P. GALLIVAN, J. R. FLANAGAN;
Queen's Univ., Kingston, ON, Canada

Abstract: Real-world action tasks, such as preparing a cup of coffee, often require searching a cluttered environment for target objects (e.g. cup, spoon) among distractors. While the top-down and bottom-up factors that influence search in real-world scenes have been well-studied, little to no work has examined whether search is influenced by the effort associated with acting upon the search target once it is found. Movement costs, such as effort, have been shown to bias decision making, both in the selection of potential movements and in perceptual decision making tasks, when greater motor costs are associated with a particular response. Here we examined if visual search can likewise be biased by motor costs associated with acting on located targets. In a series of related experiments, participants performed a search-and-then-reach task. On each trial, participants searched a display consisting of target and distractor objects and moved a cursor, controlled by the handle of a robotic manipulandum, to a target once found. Objects were chosen such that their discrimination (target vs. distractor) required fixation, and thus eye movements allowed us to assess where participants were searching. Following an initial baseline period, used to establish any pre-existing search bias, we manipulated motor costs by applying a resistive, viscous force to the handle when the cursor was located on either the left or right side of the search environment (counterbalanced across participants). We predicted that search, as measured by gaze, would be biased toward the side with less resistive force in order to minimize the motor costs associated with reaching. Results from two experiments did not provide evidence that search was biased by motor costs, with search behaviour being unaffected by the forces applied to the hand. Follow-up experiments will evaluate the impact of both active manual search, where object identity (target versus distractor) is revealed by moving the hand to the object, as well as different mechanical loads on search behaviour.

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Poster

228. Motor Control in Primates and Humans

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Title: High-dimensional posture-space analysis for upper extremity in a proctored virtual reality game

Authors: *B. A. COHN[1], R. BARMAKI[4], K. HAYASHIDA[2], F. J. VALERO-CUEVAS[3]; [1]Dept. of Computer Sci., [2]Keck Sch. of Med., [3]Div. of Biokinesiology and Physical Therapy and Dept. of Biomed. Engin., USC, Los Angeles, CA; [4]Computer and Information Sci., Univ. of Delaware, Newark, DE

Abstract: Injuries of the upper extremity, such as a rotator cuff tears or clavicle fractures, are highly prevalent and often require months of intensive rehabilitation. Considering the long recovery period and the need for multiple extended therapeutic sessions, we propose a virtual reality (VR) intervention for acute upper extremity assessment and rehabilitation. The participant is asked to wear a lightweight VR headset and complete a series of drawing tasks by moving her hand in a 3D VR environment. As the user completes the tasks, she is gradually encouraged to extend the range of motion of her upper-extremity relative to the shoulder in a gamified, interactive environment that can be easily adjusted to create an appropriate challenge, by either the participant or clinician. While previous approaches to VR-based rehabilitation focus on movement speed or range of motion, this is a self-proctored approach that automatically quantifies the kinematic quality and dimensionality of the movements the user is capable of producing, displayed in a way that is easy for clinicians to visualize and interpret. We present our findings from a pilot study in designing virtual reality interfaces for use in physical and occupational therapy clinics, discussing the ethical, practical, and experiential considerations of at-home and in-clinic motor rehabilitation with VR. Furthermore, we compare the user experience between the current practice of manual goniometric assessments to that of our VR system for both the clinician and the participant. Our results suggest that the ease-of-use and kinematic data from this VR system creates enables personalized treatment towards optimizing rehabilitation.

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Poster

228. Motor Control in Primates and Humans

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Title: Monkeys choke under pressure

Authors: *N. PAVLOVSKY[1], A. D. DEGENHART[1], P. MARINO[1], A. P. BATISTA[1], S. M. CHASE[2,3];
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Abstract: When performing in high-stakes situations, humans sometimes exhibit a frustrating phenomenon: choking under pressure. In most situations, humans perform tasks better with increasing rewards. But once rewards get too high, performance paradoxically decreases and we choke under pressure. The reason why we choke is unclear, and furthermore, the neural basis of choking is not known. We can gain insights into its neural underpinnings by demonstrating choking behavior in an animal model. Here we show that Rhesus macaques choke under pressure. We trained two animals to perform a challenging delayed reaching task. We made the task challenging by requiring the animals to make fast reaches to small targets. This pushed the natural speed-accuracy tradeoff that exists in motor behavior. At the beginning of each trial, we cued the amount of liquid reward that the animals would receive for successfully completing the task. Reward sizes were cued by the shape or color of the reach target, and they were randomly interleaved. We used four different reward sizes: small (0.1 mL), medium (0.2 mL), large (0.3 mL), and jackpot (2.0 mL). We presented the jackpot reward on only 5% of trials and evenly distributed the small, medium, and large rewards on the remaining 95% of trials. We analyzed success rate as a function of reward size. As reward size increased, both animals showed an increase in performance through the large reward. However, they exhibited a marked decrease in performance for the jackpot reward. This drop in performance on the jackpot reward trials defined the choking phenomenon. We next assessed how reward affected performance at the

delay and reach periods of the task. We calculated the percentage of trials that successfully completed each task period, and we found a choking effect during both. Moreover, choking was present in 85% of daily sessions, making it a robust effect. In one animal we assessed performance on a second task that emphasized precision over speed. Again, we found a significant choking effect. Thus, choking is a reliable effect in monkeys as seen in two animals, in many sessions, and in two different types of challenging reaching tasks. By showing that monkeys choke under pressure, we established an animal model with which to study the neural correlates of this phenomenon. Studying how choking under pressure emerges from the activity of neural circuits will help us to eventually determine the reason why we as humans choke.

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Poster

228. Motor Control in Primates and Humans

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Topic: E.04. Voluntary Movements

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Title: How does reward of the goal target modify reaching movements?

Authors: *A. DE COMITE, F. CREVECOEUR, P. LEFEVRE;
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Abstract: To control upper limb reaching movements, the central nervous system uses sophisticated strategies that rely on sensory feedbacks to produce responses to perturbations applied to the limb. These control strategies can quickly respond to mechanical perturbations in 50ms and to visual perturbations in 100ms. Current models are based on optimal feedback control, which uses a cost function that depends on various parameters such as the shape of the goal target, the presence of obstacles, etc. Implicitly, these cost functions capture the hypothesis that planning and control strategies are intended to maximize movement reward. However, possible influence of explicit target reward has not yet been documented. To address this question, we sought to determine whether and how reward is taken into account during visually guided reaching movements. More specifically, we studied whether reward of the goal target could induce changes in the corrective responses to mechanical perturbations and in movement planning. This question was addressed by varying the explicit reward of the goal target across trials. Participants performed forward reaching movements to a visual target of different values, which corresponded to the scores that participants could obtain if their movement was

successful. Movement success was determined based on whether they reached the target within a prescribed time window. During movements, constant lateral mechanical perturbations could be applied to the hand of the participants to study their feedback control strategy in detail. The presence and direction of perturbations were randomized within each block. We analyzed movement and surface electromyography of shoulder flexor and extensor muscles. Maximal hand deviation during movement in perturbed trials showed significant dependency on the reward, such that reaching to a target with larger reward induced smaller hand deviation and inversely. We also observed that maximal hand velocity was larger for reaching high rewarding targets than small rewarding ones. Surface EMG measured during unperturbed trials showed a larger baseline muscle activity for targets with a larger reward. Furthermore, muscle responses to perturbations were also larger for these targets suggesting that an increase in target value evokes an increase in feedback gains. In all, our results highlighted that explicit target rewards modulated the control gains, with a clear impact on hand speed and feedback responses to disturbances. This increase in control gains may reflect the way healthy humans mitigate the risk to miss movement goals dependent on its reward.

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Poster

228. Motor Control in Primates and Humans

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Title: Dynamical organization of the effective network via beta-gamma oscillations within the prefrontal cortex during the visual-motor mapping task

Authors: W. NIU[1], J. HYLTON[2], S. CHOU[2], R. C. SAUNDERS[2], A. R. MITZ[2], D. PLENZ[2], ***S. YU**[1,2];
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Abstract: The prefrontal cortex (PFC) plays an essential role in coordinating different brain areas in various high-level cognition processes. But little is known about the organization of mesoscopic network within PFC that can support the flexible role it plays during different types of information processing. Here we analyzed the local field potential (LFP) data collected from the PFC using chronically implanted microelectrode arrays when two macaques performed the visual-motor mapping task. We found that the PFC network exhibited swift transitions in the

oscillation frequencies across different epochs during a single trial. Specifically, 20-40 Hz oscillations dominated during the period of motion execution, which produced effective networks within the PFC that can be detected by spectral Granger causality (GC) analysis. Moreover, we found that such effective networks were constrained by distance among individual network nodes, and at the same time, can be modulated by behaviorally relevant information. Based on the simulation of a mean-field network model, we suggested that phase-lagged inter-areal inputs to the PFC may account for the observed results. Together, this study demonstrates the dynamical organization of the effective network via beta-gamma oscillations within the PFC, which may support the versatile role played by the PFC through flexible interactions with different brain areas.

Disclosures: W. Niu: None. **J. Hylton:** None. **S. Chou:** None. **R.C. Saunders:** None. **A.R. Mitz:** None. **D. Plenz:** None. **S. Yu:** None.

Poster

228. Motor Control in Primates and Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 228.14/N7

Topic: E.04. Voluntary Movements

Support: DFG, SPP 1772 Grant He7105/1.1

Title: Dimension of distality: Body-, tool- and effect-related separations of motor memories

Authors: *L. LANGSDORF[1], R. SCHWEEN[1], J. A. TAYLOR[2], M. HEGELE[1];
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Abstract: Contextual cues play a fundamental role in selecting appropriate and adapting actions for a given situation or environment. For example, different tools will have different action-outcome associations and the contextual features of these tools must cue the appropriate sensorimotor transformation for their successful operation. Previous studies suggest that only cues directly related to the state of the body, like the selected limb in a reaching task[1,2], establish context-dependent sensorimotor memories. However, modern tools have made it clear that people can successfully remember different sensorimotor transformations even though the tools share the same body state. To understand how different contextual cues can evoke the memory of different sensorimotor transformations, we presented participants three sets of contextual cues: 1) visually different cursors, 2) different action effects on the target (exploding or painting), 3) separate hands. These cues were associated with two opposing constant visual cursor rotations, respectively. Participants thus had to differentiate the relationship between cue and transformation and compensate for the perturbations. Separate memories were established by cue-dependent aiming strategies for all cues. In line with plan-based generalization[3], implicit

aftereffects showed adaptation to both rotations when tested to different target directions but were only cue dependent if the cue was related to the body state, i.e. separate hands. We therefore speculate that while aiming strategies can utilize any cue to separate the different motor behaviors to solve the dual adaptation task, implicit adaptation appears to require cues related to the state of the body. This distinction may suggest differences in credit assignment to the state of the body or the environment.[1]Howard, I. S. et al. 2013, J. Neurophysiol. [2]Seidler, R. D. et al. 2001, Behav. Brain Res. [3]Schween, R. et al. 2018, J. Neurophysiol

Disclosures: L. Langsdorf: None. **R. Schween:** None. **J.A. Taylor:** None. **M. Hegele:** None.

Poster

228. Motor Control in Primates and Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 228.15/N8

Topic: E.04. Voluntary Movements

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NIH R01-HD087089
NSF GRFP

Title: Input shaping and submovements to control dynamically complex objects

Authors: *D. E. S. DA SILVA[1], S. BAZZI[2], D. STERNAD[3], N. HOGAN[4];
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Abstract: Humans are remarkably adept at using tools and interaction with objects in general. Studies of human motor neuroscience related to physical interaction have primarily focused on the manipulation of rigid objects, like a screwdriver. However, humans are also skilled at manipulating non-rigid objects with their own complex internal dynamics, like carrying a sloshing cup of coffee without spilling. The manipulation of such dynamic objects is more challenging and unlikely to be understood by studying reaching movements or rigid object manipulation. Here, we present a study inspired by the transport of a cup of coffee without spilling to understand what strategies humans use to control external objects with internal degrees of freedom, especially in quick movements, where object dynamics dominate and sensory feedback is less useful. Subjects manipulated a simplified 2D cart-and-pendulum system in a haptic virtual environment via a robotic manipulandum, where the cart represented the cup

and the bob of the pendulum emulated liquid inside the cup. In the task, subjects were directed to move the cart from a starting point to a target point while minimizing residual oscillations of the pendulum. Several strategies for rest to rest movements of non-rigid objects have been proposed previously in the literature. All these models were optimization-based, seeking to minimize a cost function associated with a particular movement trajectory. A simpler solution to the task, known as Input Shaping, has been developed in the control theoretic literature. This technique relies on convolving a desired movement trajectory with an appropriately scaled sequence of two impulses to cancel out oscillations. The simulated movement profile is similar to the human profiles generated by an overlapping sequence of submovements. We hypothesized that humans used a strategy similar to Input Shaping to complete the task. The velocity profiles of subjects' movements were compared to the profiles predicted from Input Shaping and two optimization-based models to determine which control model best fits human performance. We also investigated how decompositions of trajectories into submovements relate to task performance. Preliminary results showed that subjects used submovements to perform the task. The amplitudes and timings of these submovements correlated with success in the task, as predicted by Input Shaping theory. Moreover, the submovement-based model was a better fit with the subjects' behavior than the optimization-based models. These results suggest that the combination of primitives, submovements in this case, is a competent description of human performance in this task.

Disclosures: D.E.S. da Silva: None. **S. Bazzi:** None. **D. Sternad:** None. **N. Hogan:** None.

Poster

228. Motor Control in Primates and Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 228.16/DP10/N9

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: E.04. Voluntary Movements

Support: NIH T32 AG052375
NIH COBRE P20GM109098

Title: Functional electrical stimulation helps support the weight of the arm during reaching and grasping movements

Authors: *A. B. THOMAS[1], R. L. HARDESTY, JR[2], A. ADCOCK[1], C. L. BRANDMEIR[1], V. GRITSENKO[3];
[2]Neurosci., [3]Human Performance - Physical Therapy, [1]West Virginia Univ., Morgantown, WV

Abstract: A common result of stroke is weakened muscles of the arm, including those that support the shoulder joint. Weakened shoulder muscles often lead to partial dislocation of the glenohumeral joint due to the force of gravity. This shoulder subluxation, in turn, causes further complications that limit the recovery of motor function including limitations in hand function. However, recent studies have shown that reducing the abduction load on the shoulder positively affects paretic hand function, possibly by reducing the overall effort needed to support the arm against gravity. While these studies used a robotic support system, functional electrical stimulation (FES) offers an alternative solution by directly activating muscles. FES is used widely in physical therapy for treatment of shoulder dysfunction after stroke. However, the effect of shoulder FES on distal limb coordination is not well understood. Here, we tested the feasibility of supporting the weight of the arm against gravity using FES and examined its effect on reaching and grasping movements.
Subjects performed unconstrained reaching and grasping movements to visual targets in a virtual reality environment with detailed visual feedback of hand position. During movement, FES was applied to the medial and anterior deltoid in ramp sequences to recreate the gravitational torque profiles of each movement. Kinematics of the arm and hand were captured using an active motion capture system, while muscle activity of the arm and hand were captured using surface electromyography. Our preliminary results in healthy, young participants show that FES is capable of assisting with shoulder abduction. The FES assistance was maintained throughout the multiple repetitions of reaching movements over a 40-minute period, which suggests that muscle fatigue can be managed with intermittent FES. The kinematics of elbow and hand motion show adaptation to the assistive action of FES. Thus it is feasible to use this gravity-assist FES technique to treat shoulder subluxation while simultaneously improving arm function in stroke survivors.

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Poster

228. Motor Control in Primates and Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 228.17/N10

Topic: E.04. Voluntary Movements

Support: NSERC Discovery Grant

Title: The extent of visuomotor adaptation depends on one's awareness of the perturbation, independent of reaching errors experienced

Authors: *D. O. WIJEYARATNAM, R. D. BISHOUTY, Z. CHENG-BOIVIN, E. K. CRESSMAN;
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Abstract: Previous work has consistently demonstrated that movements are adapted in response to a visuomotor perturbation (i.e., rotated hand-cursor feedback), regardless of whether the cursor-perturbation is introduced abruptly or gradually, and hence, large or small reaching errors experienced. Less consistent are findings related to the extent of reach adaptation as a function of the perturbation schedule (i.e., abruptly or gradually), with some reporting no differences between perturbation schedules (Klassen, Tong and Flanagan, 2005), while others report greater adaptation following reaches with a gradually introduced visuomotor perturbation compared to an abruptly introduced perturbation (Saijo and Gomi, 2010). In the current experiment, we asked if awareness may govern the extent of visuomotor adaptation achieved, independent of how the cursor perturbation is introduced. Thirty participants were divided into 2 groups, one reached with a cursor whose trajectory was immediately rotated 45° CW relative to hand motion (Abrupt Group), and the other reached with a cursor whose trajectory was gradually rotated 45° CW relative to hand motion (Gradual Group - rotation increased by 1° increments every trial up to a maximum of 45°). Participants performed 3 blocks of 150 reaching trials: Baseline (aligned cursor), Reach Training (rotated cursor) and Washout (aligned cursor). Participants in both groups were designated as aware or unaware of the visuomotor perturbation based on a questionnaire and performance in a drawing task, in which participants were asked to draw the path their hand had to make in order to get the cursor to the target. Results revealed that the planning and execution of movements early on during Reach Training were influenced by how the visuomotor perturbation was introduced (e.g., participants in the Abrupt Group took longer to initiate and execute their movements and had greater movement errors compared to participants in the Gradual Group). However, by the end of training, both groups reached in a similar manner. For reaches during Washout, analyses revealed that participants who were aware of the perturbation during Reach Training showed smaller aftereffects (i.e., smaller reach errors), compared to participants that were unaware of the visuomotor perturbation, regardless of how the cursor perturbation was introduced. Together, these results suggest that while initial visuomotor adaptation is dependent on how the perturbation is introduced, the extent of visuomotor adaptation is dependent on one's awareness of the perturbation. Participants that are unaware of the visuomotor perturbation demonstrate greater visuomotor adaptation.

Disclosures: D.O. Wijeyaratnam: None. **R.D. Bishouty:** None. **Z. Cheng-Boivin:** None. **E.K. Cressman:** None.

Poster

228. Motor Control in Primates and Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 228.18/N11

Topic: E.04. Voluntary Movements

Title: Dominant limb selection frequency increases under restricted movement planning time

Authors: *A. PRZYBYLA[1], S. AKPINAR[2], R. L. SAINBURG[3];
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Abstract: We previously showed that limb selection in targeted reaching task when given choice is associated with model of motor lateralization, which attributes dominant left hemisphere specialization to efficient coordination of limb and task dynamics. For example, we found that this motor behavior of hand selection can be modified instantaneously by occluding visual feedback during movement, as well as through a long-term unimanual athletic training such as fencing. Our findings suggested that limb selection might be driven by kinetic costs, which should place heavy demands on the planning process. We now ask if restricting the time available for movement planning would affect the pattern of limb selection behavior. One group of right-handed participants was given the choice of limb selection and two other groups were forced to move with either right or left arm to each of 32 randomly presented targets covering frontal space. Whereas in previous studies we gave the subject unlimited time, here we gave an imperative 'go' signal, upon which the subject was required to move to the target. We found that restricting time for movement planning led to significantly increased frequency of the dominant limb selection for targeted reaching, in particular to contralateral hemispace. These findings suggest that in this task the limb selection does not vary with kinetic costs, thus it is not associated with interlimb differences in sensorimotor performance identified in the model of motor lateralization. Furthermore, we found that the reaction time of the dominant limb did not vary between choice and forced condition, but intriguingly, there was substantial increase in choice condition for the nondominant limb, which in forced condition had actually shorter reaction time than the dominant limb.

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Poster

228. Motor Control in Primates and Humans

Location: Hall A

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Program #/Poster #: 228.19/N12

Topic: E.04. Voluntary Movements

Support: American Heart Association Award #18POST34060183

Title: Eye movements necessary for perceptual decisions interfere with eye-hand coordination during rapid reaching

Authors: *D. A. BARANY, M. SCHRAYER, A. M. GOMEZ GRANADOS, T. SINGH;
Univ. of Georgia, Athens, GA

Abstract: Goal-directed reaching toward visual targets requires precise integration of target, eye, and hand information to dynamically plan and update a movement. The oculomotor system plays a critical role in both facilitating visuomotor transformations for reaching and for processing visual inputs for perceptual decision-making. Previous evidence suggests overlapping neural systems underlie oculomotor responses for decision processing and eye-hand coordination, yet the behavioral consequences of this shared processing remain unclear. To address this question, we developed a rapid sensorimotor decision-making task that recruits the oculomotor system in both deciding the correct action and providing retinal and extraretinal signals to guide an appropriate motor command. We recorded limb and ocular kinematics as participants (N=21, 13 F, 22.8 ± 3.1 years) used a robotic manipulandum to perform right-hand reaching movements in a horizontal plane in response to visual targets on a virtual display. On each trial, a single target shape (circle or ellipse) appeared on either the right or left side of the visual workspace and remained in the same position (Static) or moved horizontally across the workspace at a constant Euclidean velocity (Moving). In the No-Decision (ND) condition, the target was always the same shape, and participants were instructed to hit the target as quickly and as accurately as possible. In the Decision (D) condition, the shape of the target cued the participants to either hit the target or reach to a pre-specified location away from the target. We found that added decision resulted in a lower success rate (D: 85 ± 10%; ND: 95 ± 5%), longer hand reaction times (D: 431 ± 59 ms; ND: 260 ± 39 ms), and less accurate initial movement directions (D: 31 ± 18°; ND: 24 ± 8°), suggesting that the demands of the decision disrupted the early planning period. The reach deficits were associated with distinct eye-movement strategies: saccadic reaction times in the Decision condition were faster (D: 174 ± 11 ms; ND: 182 ± 17 ms) but less accurate (D: 35 ± 14 mm; ND: 31 ± 13 mm), indicating that participants were prioritizing target identification over spatial location information. During Moving trials, smooth pursuit durations were longer (D: 474 ± 50 ms; ND: 280 ± 61 ms) and exhibited a higher frequency of catch-up saccades (D: 0.07 ± 0.02 saccades/s; ND: 0.04 ± 0.02 saccades/s), reflecting lower quality target tracking during ongoing decision processing. Together, these results suggest that limb performance deficits during a simultaneous decision-making and reaching task are in part due to less reliable oculomotor behavior relevant for accurate visuomotor transformations.

Disclosures: D.A. Barany: None. **M. Schrayer:** None. **A.M. Gomez Granados:** None. **T. Singh:** None.

Poster

228. Motor Control in Primates and Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 228.20/N13

Topic: E.04. Voluntary Movements

Support: University of Georgia Research Foundation

Title: Oculomotor deficits in older adults may contribute to deficits in perceptual decision-making and limb motor performance in a rapid sensorimotor task

Authors: *A. M. GOMEZ GRANADOS, D. A. BARANY, M. SCHRAYER, T. SINGH; Univ. of Georgia, Athens, GA

Abstract: Concurrent declines in perceptual, motor and cognitive processes in older adults contribute to decline in eye-hand coordination. However, how deficits in interactions between these processes affect functional movements is still unclear. Here, we asked how perceptual decision-making under time-pressure affects visuomotor performance. Young and older participants (20-30 and 66-73 years old) performed manual interception and reaching movements to objects in the peripersonal space while we measured their limb and ocular kinematics. The task was performed using a KINARM Endpoint robot (BKIN Technologies). Participants had to discriminate between two possible objects (circle or ellipse), and were instructed to reach towards the circles (target) and avoid the ellipses. They were instructed to hit the target as quickly and accurately as possible. Each trial presented a single object (circle or ellipse) that would either remain in the same position (Static Condition) or would move across the screen horizontally (Moving Condition). In the Decision condition participants were presented with either of the two objects in randomly interleaved trials. In the No-Decision condition participants were presented only with circles (targets). Older adults exhibited longer reaction times and lower accuracy than young adults (mean±sd reaction time: 396.1±113.5 ms vs 345.6±99.4 ms, accuracy ratio: 0.75±0.15 vs 0.9±0.09). Both groups were more accurate in the Static condition and in the No-Decision condition. Older adults made the initial saccades after object appearance later than young adults (mean±sd 231.1±45.3 ms vs 178.2±14.8 ms). In both age groups, saccades occurred sooner in the Decision condition than the No-Decision condition. The initial saccade distance error (distance between the target and where the first saccade landed) shows that older adults landed the first saccade farther away from the objects (mean±sd 64.3±32.7 mm vs 32.7±13.8 mm). For both groups, the saccade distance error was also greater during the Decision Condition. During the Moving condition in which objects moved across the workspace, older adults made smooth pursuit eye movements that were shorter in duration (mean±sd 294.4±94.7 ms vs 376.8±112.7 ms); they also made more catch-up saccades. Total Smooth-Pursuit time and number of catch-up saccades were higher for both age groups in the Decision Condition. Together, these results suggest that deficits in oculomotor performance may contribute to deficits in perceptual-decision making and limb motor performance and these deficits may exacerbate overall performance in older adults.

Disclosures: A.M. Gomez Granados: None. **D.A. Barany:** None. **M. Schrayer:** None. **T. Singh:** None.

Poster

228. Motor Control in Primates and Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 228.21/N14

Topic: E.04. Voluntary Movements

Support: JST CREST (JPMJCR14E4)
JSPS KAKENHI (JP16H06566)

Title: Distinct temporal frequency-dependent modulations of direct and indirect visual motion effects on reaching adjustments

Authors: *H. UEDA, N. ABEKAWA, S. ITO, H. GOMI;
NTT Communication Sci. Labs., Atsugi, Kanagawa, Japan

Abstract: We have previously shown two distinct visuomotor responses in visually guided reaching: the fast direct effect of visual motion analysis to generate quick reactions and the relatively slow indirect effect of visual motion analysis to induce motor adjustments via position representation (Ueda et al. 2018). From the temporal feature of these responses, we hypothesized that the initial response is generated by the reflexive visuomotor mechanism and the subsequent one is coupled with the visual processing for perception.
Here, to test this hypothesis, we examined the influence of visual motion with different temporal frequencies on these initial and late responses during visually guided reaching. Since reflexive sensorimotor responses and motion perception are known to have different sensitivity tunings depending on spatiotemporal frequency (Gomi et al. 2006), the initial and late phase of the adjustment during visually guided reaching should show different modulation according to their distinct sensitivities.
In the experiment, participants performed reaching movements toward a target patch which had either a sharp or a fuzzy edge. A sinusoidal grating pattern was presented inside of target stimulus. Immediately after the reaching initiation, the grating started to drift in a random horizontal direction with a temporal frequency of 6 or 12 Hz. A target shift was also introduced in randomly selected trials, and participants were required to make online reaching adjustments in response to the target shift. The size of the adjustment response was defined by the hand acceleration orthogonal to the reaching direction. Since motion signals strongly interact with position representation when position uncertainty is high (Ueda et al. 2018), the effect of motion-induced position shift can be extracted by subtracting the response to the sharp edge target from the response to the fuzzy edge target.
As expected, the results ($N = 21$) showed that the initial response was significantly greater for the higher temporal frequency stimulus ($p < 0.01$) while the latter interaction effect was reversed ($p < 0.01$). This double dissociation strongly supports the idea that visually guided reaching

adjustment is generated by two distinct visuomotor processes: one relies on the direct visual motion analysis and the other relies on the position representation which is affected by visual motion. The slow build-up of the interaction effect is reasonable in terms of computational complexity, as additional computations are needed to integrate motion and position information. The direct effect, on the other hand, would be necessary for successful interaction with dynamic environments.

Disclosures: H. Ueda: None. **N. Abekawa:** None. **S. Ito:** None. **H. Gomi:** None.

Poster

228. Motor Control in Primates and Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 228.22/N15

Topic: E.04. Voluntary Movements

Support: BSF 2015327

Title: Prolonged reaction times for curved trajectories are associated with delayed initiation of the motor planning

Authors: *L. SHMUELOF[1], G. FEINSTEIN[2];
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Abstract: The processes of issuing a motor movement can be described as a three stage process: 1. Perception of stimuli 2. Action selection 3. Motor planning. While the effect of action selection on reaction time (RT) is well established, the reaction time costs and neural substrates associated with motor planning are not well known. We hypothesized that unlike planning of straight point-to-point movements, where the trajectory is likely to be an automatic outcome of a control policy, when subjects are required to perform a curved trajectory, they have to generate a kinematic plan of the trajectory, in a time consuming process. In a behavioral experiment, we found two evidence supporting a categorical difference between curved and straight trajectories. The first is an additional reaction time (RT) for the issuing of curved compered to straight trajectories and the second is a switching cost between issuing these two types of trajectories. Interestingly, in the experiment in which the switching cost was observed, the additional RT required for issuing curved compered to straight trajectories was not observed, probably due to the fact that subjects did not have explicit information regarding the possible targets in each trial. This observation points to a difference in the pre-planning strategy of straight and curved trajectories. In a follow-up slow event-related fMRI experiment, searching for the neuronal substrates of motor planning, we found two brain regions that showed increased activation for curved compared to straight trajectories during motor planning: the Inferior Frontal Gyrus (IFG)

and the Inferior Parietal Lobule (IPL). Interestingly, these areas also showed increased activation for the straight trajectories (compared to curved trajectories) during the action selection phase, when multiple optional targets were presented. Our results suggest that the RT costs associated with the planning of curved trajectories are an outcome of the fact that curved trajectories are planned after action selection whereas straight trajectory planning starts during the action selection phase, when multiple optional targets are considered.

Disclosures: L. Shmuelof: None. **G. Feinstein:** None.

Poster

228. Motor Control in Primates and Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 228.23/N16

Topic: E.04. Voluntary Movements

Support: NSERC

Title: Representational dissimilarity analysis of EEG patterns reveals the time course and computational hierarchy of grasp features during grasp planning and execution

Authors: *L. GUO[1], Y. SHAMLI OGHLI[1], A. FROST[1], M. NIEMEIER[1,2];
[1]Univ. of Toronto, Toronto, ON, Canada; [2]The Ctr. for Vision Res., York Univ., Toronto, ON, Canada

Abstract: The seeming ease with which we reach to grasp objects is supported by complex neuro-computational mechanisms. As a critical landmark of the computations, the brain needs to integrate the effectors that will be used to execute the action with other motor features of the grasp program (e.g., grip orientation). Previous work has shown that grasp goal representations emerge upstream from effector specification. However, these studies probed grasp goal representations at a rather coarse level. To study grasp goal representations at a finer level, here we asked participants to grasp objects with different effectors (left, right, or both hands), using two different grasp orientations. Each trial began with a Preview of the object followed by an auditory Go signal. Crucially, grasp orientation was instructed prior to each block of trials while effector choice varied from trial to trial specified by the Go signal. We inferred representations of grasp features and their timecourse by applying time-resolved representational dissimilarity analysis to EEG patterns. Our results showed that orientation representation before and after effector specification display different properties, and more importantly, that a robust orientation representation depends on effector specification. Further analyses showed that left hand and bimanual grasps shared overlapping representations, consistent with findings that both grasps involve the right anterior intraparietal sulcus. Together, these results reveal the timecourse and

representational hierarchy of grasp features over the course of planning and executing precision grasps.

Disclosures: L. Guo: None. **Y. Shamli Oghli:** None. **A. Frost:** None. **M. Niemeier:** None.

Poster

229. Sensorimotor Learning I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 229.01/N17

Topic: E.04. Voluntary Movements

Title: Does latency of motor-evoked potentials influence changes in decreased short-interval intracortical inhibition and scores of force control practice?

Authors: *A. MARUYAMA[1,2], D. SATO[2], K. YAMASHIRO[2], K. KURIHARA[2], I. TOCHIKURA[2], Y. SUEYOSHI[1], A. NURUKI[1], S. ETOH[1], M. HAMADA[3];
[1]Kagoshima Univ., Kagoshima, Japan; [2]Hlth. and Sports, Niigata Univ. of of Hlth. and Welfare, Niigata, Japan; [3]The Univ. of Tokyo, Tokyo, Japan

Abstract: The efficiency of late I-wave recruitment and after-effects of TBS protocols are highly variable among individuals. These after-effects are correlated with the ease of late I-wave recruitment, which represents the variability of the latency of MEP evoked by TMS pulses that induce anterior-posterior (AP) and latero-medial (LM) directed currents (Hamada et al., 2013). This finding suggests that the wide range of synaptic plasticity responses caused by the after-effects of TBS is influenced by the ease of late I-wave recruitment. It seems that the short-interval intracortical inhibition (SICI) by a paired-pulse TMS decreases after motor skill practice and motor control training. The decreased SICI leads to task-dependent cortical plasticity in the motor cortex. We investigated whether the sensitive ease of latency of MEP induced by TMS pulses in the motor cortex could affect the variable degrees of SICI disinhibition and the individual difference in scores of short-term pinch force control practice.
Performance of force control tasks was expressed in the integral values of errors to subtract actual pinch force from the targeted tracking force, coordinating with the right-handed FDI and APB around muscles. The tasks comprised sine curves of 0.3 and 0.5 Hz and 4 force intensities of 5-30% of MVC. All participants performed 6 sets of force control tasks separated by 3 minutes of rest. Each set consisted of 4 tasks of 20 seconds' duration.
The onset latency of MEPs using PA, AP, and LM currents was measured during mild contraction of the target muscle by Hamada et al. (2013). SICI (3-ms interval) and SICF (1.5-ms interval) provided by a paired-pulse TMS were assessed before practice and immediately after (0), 5, 10, and 20 minutes of recovery time (RT) of practice, based on the Kujirai and Ziemann paradigms (1996, 1998).
The latency of MEPAP-LM differences significantly correlated with the percentage changes of

$SICI_{RT}$ to $SICI_{baseline}$, and the under and absolute errors significantly correlated with percentage changes of $SICI_{RT}$ to $SICI_{baseline}$, respectively. Hence, the longer the duration of AP-LM latency, which is limited to some ranges of latency of MEP, the greater the percentage of SICI changes disinhibited after motor force control. The variable scores of force control tasks were related to the variability of SICI disinhibition. Thus, it is possible that the ease of late I-wave recruitment influences individual differences in the performance of motor force control.

Disclosures: A. Maruyama: None. **D. Sato:** None. **K. Yamashiro:** None. **K. Kurihara:** None. **I. Tochikura:** None. **Y. Sueyoshi:** None. **A. Nuruki:** None. **S. Etoh:** None. **M. Hamada:** None.

Poster

229. Sensorimotor Learning I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 229.02/N18

Topic: E.04. Voluntary Movements

Title: Elimination of internally generated errors due to motor output noise from the signal driving motor adaptation

Authors: *T. RANJAN[1], M. A. SMITH[2];
[2]Sch. Engin., [1]Harvard Univ., Cambridge, MA

Abstract: Motor learning is largely driven by errors in our actions, which are generated either internally due to motor output noise, or externally due to perturbations from the outside environment. If adaptation were driven by overall motor error, i.e., the sum of internally-generated (IG) and externally-generated (EG) errors, the ability to adapt to externally-generated environmental perturbations would be muddied by adaptation from internally-generated motor output noise. Here we show that although the motor system cannot stop motor noise from occurring, it can cancel the effect that it would bring to error-dependent motor learning so that this learning is not corrupted by it. To determine if IG & EG motor errors have different effects on motor adaptation, we designed an adaptation paradigm where the EG errors were matched with the IG errors in magnitude and temporal structure. In experiment 1, we delivered pseudorandom zero-mean visuomotor rotation (VMR) perturbations (of 0^o, $\pm 2^o$ & $\pm 4^o$) such that the RMS perturbation size closely matched the RMS error in movement direction observed in unperturbed trials in pilot data (~2.5^o in both cases). We first examined the effect of EG errors on adaptation, and unsurprisingly found an approximately linear adaptive response to the small external perturbations, with an average adaptation rate of 0.24. To examine the effect of IG errors, we dissected the net error following each perturbation into a perturbation induced EG error and a motor noise induced IG error. Surprisingly, we found that the adaptation rate to IG errors was far smaller than that for EG errors (0.01 vs 0.24). In a 2nd experiment, we tested the

possibility that the proprioceptive-visual (PV) mismatch inherent to EG VMR perturbations might drive the apparent difference in learning from EG vs IG errors observed in expt 1. When we eliminated the PV mismatch by perturbing visual and proprioceptive feedback identically using physical force field (FF) perturbations, we again found adaptation rates that were much smaller for IG vs EG errors (0.1 vs 0.23). Together, our results indicate that the motor system deftly parses out motor errors into internally- and externally-generated parts, and specifically uses the externally-generated contribution to drive motor adaptation.

Disclosures: T. Ranjan: None. **M.A. Smith:** None.

Poster

229. Sensorimotor Learning I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 229.03/N19

Topic: E.04. Voluntary Movements

Title: Reinforcement does not create a new baseline

Authors: *J. MAGNARD[1], J. CESONIS[2], R. SCHWEEN[3], R. L. SAINBURG[4], D. W. FRANKLIN[2], N. SCHWEIGHOFER[1];
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Abstract: The motor system rapidly adapts to external perturbations (*e.g*., manipulation of visual feedback) through the update of an internal model based on sensory prediction errors. However, newly learned mappings are quickly forgotten. By studying the decay of visuomotor adaptation through a visual error clamp design, Shmuelof et al. (2012) showed that a reinforcement phase following adaptation featuring binary feedback without sensory prediction errors enhanced retention. They suggested that reinforcement creates a new baseline towards which the newly acquired memory decays. Our objective was to extend these results by testing whether the new baseline was the result of forming a new memory via a reinforcement learning process or of a decrease in decay rate via reinforcement. *Methods*. The original protocol of Shmuelof et al. was run both at the University of Southern California (n = 30), with a Kinereach air-sled system, and at the Technical University of Munich (n = 20), with a vBOT 2D planar robotic system. The arm reaching movement task was composed of 6 blocks with a target located at 135°: a baseline block, a 30° CCW rotation of the cursor's direction block, an asymptote block (80 trials), a 45° CCW rotation block, a visual error clamp block (with the visual cursor's direction error = 0°), and a washout block. A Binary Error + Vector Error (BE+VE) and a Binary Error (BE) group both received binary auditory and visual cursor feedback, except during the asymptote block, where cursor feedback was withheld for the BE group. A second protocol (n = 45) was designed

to enhance the original effects shown by Shmuelof et al. with a combination of 1) a longer asymptote (160 trials); 2) a no-feedback block instead of the error-clamp block; and 3) a 15° instead of a 45° CCW rotation after asymptote. In addition, we omitted the auditory reward from non-asymptote blocks. Finally, besides BE+VE and BE groups, a new Vector Error (VE) group, which received only online vector error during the asymptote block, was included. *Results*. For all three experiments, our results showed the same decay rate between groups during the visual error clamp/no-feedback block, which illustrated a rapid reversion towards a value intermediate between the reinforced action and baseline performance. *Discussion*. Our results did not replicate the original findings of Shmuelof et al. The exposure to only binary feedback after initial adaptation did not turn the adapted state into a new baseline, even when we increased the length of the reinforcement block. To conclude, our data do not support reinforcement of learned actions preventing motor forgetting.

Disclosures: J. Magnard: None. **J. Cesonis:** None. **R. Schween:** None. **R.L. Sainburg:** None. **D.W. Franklin:** None. **N. Schweighofer:** None.

Poster

229. Sensorimotor Learning I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 229.04/N20

Topic: E.04. Voluntary Movements

Support: JSPS Grant 17H00874

Title: Encoding the control position relative to hand in motor memory for manipulating a tool

Authors: *H. HARA[1], M. TAKEMI[1,2], S. HAGIO[1], D. NOZAKI[1];
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Abstract: Tools can be a physical extension of the limbs allowing us to reach farther objects and to manipulate objects more dexterously. Recently, Heald et al. (Nat Hum Behav 2018) have demonstrated that the motor system could develop distinct motor memories when control points on a tool were different. This is reasonable, because forces and/or torques (i.e., motor commands) required to move a rigid-body tool should vary with the control points. The remaining questions are 1) if the motor memories for different control points are inherently different (or acquired through motor adaptation process) and 2) if so, how the motor memories encode the spatial information of control points.
The current study addressed these questions by investigating how the motor adaptation effect acquired by moving a particular point was generalized when controlling different points. Considering that required motor commands for nearer control points are more similar than those for farther points, we expected the motor adaptation effect gradually decreased as the control

points became more distant from the original training point.
Participants moved a rectangular virtual tool (2 cm height x 20 cm width) with their right arm while holding a robotic manipulandum. The hand position was always fixed at the center of tool. There were 7 visual control points over the tool (0, ±3, ±6, ±9 cm lateral from the hand position). Participants were asked to reach one of the control points to the target 10 cm ahead. Notably, the physical hand movements were always identical irrespective of the control points.
A velocity dependent curl force field was imposed when the participants moved the central control point (the hand position). After adapting to the force field sufficiently, force channel trials were interleaved to quantify the aftereffects when moving different control points. The visible group (N = 10) could see the hand, while the invisible group (N = 10) could not throughout the experiment.
The aftereffects decreased with the distance of control points from the hand position in the visible group. However, the modulation was significantly weaker in the invisible group as shown by the presence of a significant interaction between control point and experimental group (two-way repeated measures ANOVA, $F(6,108) = 3.17$, $p = 0.007$). These results indicate that the motor memories inherently encode the spatial information of control points relative to the hand position and that explicit visual information of hand is necessary for the motor system to obtain the spatial information. This encoding characteristics are the foundation to provide the motor system with the flexible ability to skillfully manipulate a tool.

Disclosures: **H. Hara:** None. **M. Takemi:** None. **S. Hagio:** None. **D. Nozaki:** None.

Poster

229. Sensorimotor Learning I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 229.05/N21

Topic: E.04. Voluntary Movements

Support: Australian Research Council DP180103081
Australian Research Council FT120100391

Title: Effects of task error history and time on adaptation

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Abstract: Traditional views of sensorimotor adaptation, or adaptation of movements to perturbed sensory feedback, emphasize the role of automatic, implicit correction of sensory prediction errors (differences between predicted and actual sensory outcomes). However, latent

memories formed from sensorimotor adaptation, prominently evidenced in improved learning (i.e., savings), have recently been attributed to strategic corrections of task errors (failures to achieve task goals). To dissociate contributions of task errors and sensory prediction errors to latent sensorimotor memories, we perturbed target locations to remove or enforce task errors during learning and/or test. We show that prior learning to correct task errors was sufficient for savings: a history of sensory prediction errors was neither sufficient nor obligatory for savings. Limiting movement preparation time further revealed two distinct components of this learning: 1) time-consuming, flexible strategies, and 2) rapidly expressible, inflexible stimulus-response associations. We subsequently designed experiments to dissociate the role of task errors and sensory prediction errors on offline consolidation of sensorimotor adaptation. Results indicate that time post-task acted upon a memory of task errors, improving the capacity to flexibly improve adaptation to novel perturbations only when a history of task errors was present, and not when there was no history of task errors.

Disclosures: L. Leow: None. **E. Reuter:** None. **W. Marinovic:** None. **T.J. Carroll:** None.

Poster

229. Sensorimotor Learning I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 229.06/N22

Topic: E.04. Voluntary Movements

Support: Department of Science and Technology, Government of India

Title: Motor adaptation in response to changing movement goals

Authors: *D. P. SADAPHAL[1], A. KUMAR[1], P. K. MUTHA[2];
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Abstract: Motor adaptation has largely been studied using paradigms that perturb the actual or seen position of the limb, and appears to be driven by implicit processes, explicit strategies and operant mechanisms. In typical adaptation tasks, movement goals always remain fixed. Here we addressed the previously unexplored question of how humans adapt their movements in response to changing movement goals. We hypothesized that such adaptive behavior would be driven mainly through the development and expression of explicit strategies. Participants first made 56 “baseline” point-to-point reaching movements to fixed, stationary targets. This was followed by 112 “adaptation” trials in which the target was consistently “jumped” by a fixed magnitude in the same direction on each trial (TJ). Interspersed within the TJ trial block were 3 sub-blocks of 4 trials each in which the target was not jumped (NJ). We instructed subjects to reach to the new target on the TJ trials and the original target on the NJ trials; instructions were given every time

the jump conditions changed. Following adaptation, subjects performed 112 “washout” (NJ) trials and then a second re-adaptation block identical to the first. Subjects easily adapted to the jump perturbation; they progressively directed their movements straight to the new target on the TJ trials. Interestingly, subjects also rapidly “de-adapted” on the NJ trials; they moved directly to the original target location, a pattern that held even on the last sub-block of NJ trials. This indicated that subjects had evolved a strategy to account for the jump, which they switched off when instructed. This was also reflected in the longer reaction times on TJ trials and the complete absence of an after-effect during washout. During re-adaptation, subjects showed faster learning than naïve and rapid “de-adaptation” again, indicating substantial savings of the prior strategy. These results thus indicate that adaptation to changing movement goals occurs almost exclusively through learning of cognitive strategies, and are consistent with the view that savings emerges from the use of such strategies. Furthermore, our task and approach provide a foundation to probe the neural correlates of strategy-use in motor adaptation in the future.

Disclosures: D.P. Sadaphal: None. **A. Kumar:** None. **P.K. Mutha:** None.

Poster

229. Sensorimotor Learning I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 229.07/N23

Topic: E.04. Voluntary Movements

Support: KAKENHI 16J04573

Title: Sensorimotor adaptation to novel muscle dynamics induced by electrical stimulation

Authors: *S. HAGIO, D. NOZAKI;
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Abstract: Human behavioral system is formulated based on prediction errors estimated by comparing predicted sensory feedback with the actual that reflects the interaction between internal and external environments of a body (Shadmehr et al. 2010). Many previous studies demonstrated motor adaptation to novel external environments (Shadmehr and Mussa-Ivaldi, 1994), or movements with a new tool (Imamizu et al. 2000). However, we have to adapt movements not only to the change of external environment but also internal environment that is caused by muscle damage, pain and fatigue. Here, we examined how muscle activation is modified to adapt to novel internal dynamics in the musculoskeletal system using high-frequency electrical muscle stimulation (EMS) that makes it possible to rapidly induce fatigue in a single muscle.Participants sat in a chair and held a handle attached to six-axis force transducer by their right hand. A cursor was displayed in a screen located in front of their body, the xy-coordinates of which represented the vectors of horizontal isometric force, F_x and F_y, produced against the

handle. The experimental session consisted of baseline and EMS blocks. In the baseline block, the participants were instructed to move the cursor from a start position to a target located in a left side as straight as possible and maintain the cursor position at the target for 1.5 sec. The magnitude of force was determined based on the force during maximal voluntary isometric contraction (MVC). At the beginning of the EMS blocks, trains of rectangular and biphasic pulses (60Hz; pulse duration, 500 μsec) were applied to a long head of biceps brachii muscle (BiBrac) for 5 min. The amplitude of EMS was adjusted to produce approximately 20 % level of MVC. After the long-term EMS, the force production tasks following the EMS for 10 sec were repeated. To measure the learning response, cursor-clamp trials were randomly interleaved, with which the trajectory of the cursor was constrained to a straight path from the start position to the target. During the trials, surface electromyograms were recoded from 14 muscles spanning wrist, elbow, and shoulder joints.During the long-term EMS, the induced force was abruptly decreased to approximately 20 % of the maximum for 30 sec. Consequently, the produced force was deviated to the lateral direction from the straight path to the target, which was opposite to the mechanical direction of BiBrac. The errors were then rapidly reduced by increasing activation in synergistic muscles although only BiBrac was fatigued. The results indicate that motor commands to multiple muscles were complementarily modified to adapt to the change of internal dynamics in a single muscle.

Disclosures: S. Hagio: None. **D. Nozaki:** None.

Poster

229. Sensorimotor Learning I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 229.08/N24

Topic: E.04. Voluntary Movements

Support: DFG Project No. KL 2990/1-1
SFB 874 (No. 122679504)

Title: The relationship between implicit and explicit visuomotor task learning in hippopcampus and parietal cortex

Authors: *R. LIENKÄMPER, M. SAIF-UR-REHMAN, S. DYCK, Y. PARPALEY, J. WELLMER, C. KLAES;
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Abstract: Different brain structures are involved in learning explicit or implicit tasks. The structures mostly associated with implicit learning are the basal ganglia, parietal cortex and frontal areas, while explicit learning is believed to rely mainly on the hippocampus. However, both types of learning can interact with each other. For example, tasks that have been learned

explicitly can be transformed into an implicit representation after overlearning.
Apart from the tasks explicitness, the involvement of hippocampus and parietal cortex has also been shown to vary depending on the difficulty of a task and its novelty. However, transitions from the involvement of one structure to the other are not yet understood. Using a head-mounted virtual reality device, we are investigating the changing involvement of hippocampus and parietal cortex during a center out reaching task while recording EEG, ECoG and/or single unit activity in human subjects. The task can be done under two conditions: Visuomotor adaptation and visuomotor association. For the visuomotor adaptation, we are introducing a misalignment between visual input (the observed movement of the arm in virtual reality) and the motor output (the movement of the subject's arm in reality). Visuomotor association represents the most explicit condition, with a non-spatial cue (a Chinese number word) being shown that must be associated with one of the possible targets (which are labelled with the Chinese numbers from one to six).
These conditions form a transition from implicit (visuomotor adaptation) to explicit learning (association), with preliminary behavioural results showing that the strength of misalignment in the adaptation condition significantly impacts the path length and completion time, indicating that this can be considered a change in the task difficulty. We are trying to achieve a similar change of difficulty in the association condition by changing the numerical cue to a mathematical equation, e.g. adding two numbers.
Preliminary EEG-data shows an increase in parietal beta frequency power along the task difficulty as well as compared to the association subtask, which might hint towards a gradual change in parietal cortex involvement.

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Poster

229. Sensorimotor Learning I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 229.09/N25

Topic: E.04. Voluntary Movements

Title: Divisively normalized processing of redundant visual error information by visuomotor adaptation system

Authors: *Y. MAKINO[1], T. HAYASHI[2], D. NOZAKI[1];
[1]Grad Sch. of Edu, Univ. of Tokyo, Tokyo, Japan; [2]Sch. of Engin. and Applied Sci. Harvard Univ., Cambridge, MA

Abstract: The adverse effect of visual feedback uncertainty on motor adaptation has been explained by the Bayesian model assuming that the uncertainty makes the measurement of error

more unreliable (Burge et al., 2008; Wei & Kording, 2010). However, the mechanistic explanation of how this calculation is achieved has been largely unknown. Notably, visual feedback uncertainty is often provided as a cloud of multiple cursors. Thus, understanding the mechanisms requires elucidating how the motor system processes the redundant visual error information, which was the purpose of the present study.
Ten participants moved a cursor toward a front target with their unseen hand. In the perturbation trial, a single cursor (±30, ±22.5, ±15, ±7.5, 0 deg) or double cursor perturbations (combination of two of ±30, ±15, 0 deg) were imposed (Kasuga et al. 2013) while the hand movement was constrained with a force channel. In the next probe channel trial, the aftereffect was quantified as the lateral force. The cycle of perturbation, probe, and 2 washout trials was repeated.
We observed that the dependence of aftereffect with visual error size obtained in the single cursor condition was significantly changed by the presence of another cursor. The perturbations in the opposite directions (e.g., 30 and -15 deg) suppressed the aftereffects, implying the additive effect. However, the perturbations in the same directions (e.g., 45 and 15 deg) did not increase the aftereffects. We found that such a complicated pattern was explained by a computational model assuming that neuronal elements encode visual error with their own receptive field and the output was normalized by the summation of outputs (divisive normalization: Carandini & Heeger 2011; Hayashi et al., 2019).
The results of additional experiment (N = 9) indicated that this model successfully predicted the aftereffect when three cursors were displayed (15, 30 and one of (-30, -15, 0, 45) deg). Moreover, our model could reproduce the adverse effect of visual feedback uncertainty on the motor adaptation. Thus, the present study clarified the way of redundant visual error information processing in the motor system and provided a mechanistic insight into the problem of why the visual feedback uncertainty degrades the motor adaptation.

Disclosures: **Y. Makino:** None. **T. Hayashi:** None. **D. Nozaki:** None.

Poster

229. Sensorimotor Learning I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 229.10/N26

Topic: E.04. Voluntary Movements

Support: MRC-ARUK Centre for Musculoskeletal Ageing Research

Title: Targeted TDCS improves proximal and distal upper limb function: Evidence from motor adaptation

Authors: ***M. WEIGHTMAN**[1], J.-S. BRITTAIN[2], C. MIALL[2], N. JENKINSON[1];
[1]Sch. of Sport, Exercise and Rehabil. Sci., [2]Sch. of Psychology, Univ. of Birmingham, Birmingham, United Kingdom

Abstract: Good motor control of the upper limb is vital for carrying out even the most basic activities of daily living and therefore dysfunction in ageing- and patient- populations can impose large burdens on quality of life. Control of the proximal and distal portions of the upper limb appear to be underpinned by somewhat different neural substrates. Direct connections from the primary motor cortex (M1) to distal muscles supports the crucial role of M1 in the control of hand/finger movements. Alternatively, impaired reach behaviour after cerebellar degeneration and ataxia highlights the cerebellum as a key neural substrate contributing to whole arm reaching.
Transcranial direct current stimulation (TDCS) is a form of non-invasive brain stimulation that can be used to modify the excitability of discrete brain areas. We combined targeted stimulation of the cerebellum and M1 with tasks that shared similar motor control characteristics but isolated whole arm movements from hand/fingers movements, in order to investigate whether the distal versus proximal aspects of upper limb motor performance could be selectively modulated. Both young (19.5 ±1.4 yrs) and older (74.8 ±4.1 yrs) adults received anodal TDCS over the cerebellum, M1 or sham stimulation during a visuomotor rotation task requiring either hand/finger or whole arm reaching movements. Participants were asked to make fast centre out 'shooting' movements towards targets using either a joystick or 2D robotic manipulandum, while a 60° rotation was unexpectedly added.
Our results show that cerebellar stimulation improves adaptation for both young and older adults in the whole arm reaching task, as they displayed significantly reduced total error compared to the M1 or sham group. Reduced error was still present in older adults after the cessation of stimulation, when re-tested on the same task. Conversely, M1 TDCS significantly enhanced adaptive performance for both age groups in the hand/finger task, with no effect of cerebellar or sham stimulation. These results suggest that specific improvements in proximal and distal upper limb motor control can be achieved via targeted TDCS. Importantly, these effects remain accessible to older adults, which is crucial if TDCS is to become a clinically viable tool for neurorehabilitation of upper limb deficits in ageing and disease.

Disclosures: **M. Weightman:** None. **J. Brittain:** None. **C. Miall:** None. **N. Jenkinson:** None.

Poster

229. Sensorimotor Learning I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 229.11/N27

Topic: E.04. Voluntary Movements

Support: Ramanujan Fellowship

Title: Retention and interlimb generalization of motor skill learning have common features

Authors: *G. YADAV, P. K. MUTHA;
Indian Inst. of Technol., Gandhinagar, India

Abstract: Recent sequence learning studies suggest that the acquired memory must be in an unstable, fragile state for generalization or transfer to untrained conditions. We asked whether generalization of motor skill learning across limbs is influenced by the stability of the acquired memory. Healthy right-handed individuals learned a new motor skill task in which they were required to accurately move their hand to a target circle within 550 msec. Participants were randomly divided into two groups: 1) LR, who practiced with their left arm first followed by the right, and 2) RL, who practiced in the reverse order. Participants were tested for transfer on the other arm following a period of 24 hours. To assess transfer, we compared the naïve performance of an arm with its performance after the other arm had undergone learning (e.g., the right arm of the RL and LR groups). We found significant and symmetric transfer of learning to the untrained arm even after 24 hours, indicating that interlimb transfer is evident even after the memory has stabilized. Interestingly, the magnitude of transfer was similar to the magnitude of retention seen in two additional groups of participants who were trained and later tested at 24 hours on the same arm (RR and LL groups). We then speculated that a stabilized memory may mediate both transfer and retention of the learned skill. In order to test this idea, we performed another study in which participants (LR and RL groups) learned a novel motor skill under a variable (required to make fast and accurate movements to one of the 8 randomly presented targets) or constant (made fast and accurate movements to a single target) task conditions. Following learning, participants performed the corsi block tapping task, which impairs retention under variable but not constant task conditions. All the participants were later tested at 24 hours for transfer of the newly acquired skill on the untrained arm. We found that performing the corsi block task now also impaired interlimb transfer under the variable but not constant conditions. Taken together, these results suggest that interlimb transfer is evident even after skill memory consolidation, and that it may arise from mechanisms that also drive motor memory retention.

Disclosures: G. Yadav: None. **P.K. Mutha:** None.

Poster

229. Sensorimotor Learning I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 229.12/N28

Topic: E.04. Voluntary Movements

Support: ESA
BELSPO/PRODEX
FNRS

Title: Online changes in movement representations could be preserved in memory for at least 500ms

Authors: *P. LEFEVRE, J. MATHEW, F. CREVECOEUR;
ICTEAM and Inst. of Neuroscience, Univ. Catholique de Louvain, Louvain-la-Neuve, Belgium

Abstract: Humans adapt to mechanical perturbations within tens of trials, but how quickly this process influences motor behavior has only been recently investigated. Recent findings suggest that adaptive feedback control happens within trial, ie, corrective movements can be tuned to the specific perturbation of each individual trial. This was highlighted in our previous work with a reaching experiment in which participants had to stop at a via-point located between the start and the goal. On catch trials, we applied a force field during the first part and then unexpectedly switched it off at the via-point. Our results highlighted an after effect when participants exited the via-point in less than 500ms, which is consistent with standard adaptation scenarios. These previous observations raised the question of whether the after-effect evoked within the sequence was similar to standard sustained and long-lasting after-effects, or whether it would be rapidly forgotten because of the fact that it resulted from transient disturbances. In such case, as we are studying reaching adaptation in a very rapid time scale, one could expect that the movement corrections would be lasting for a short time. The aim of the study was to investigate how long changes in movement representations lasted within the trial. For this, we have studied the same reaching task through a via-point on the pathway in two situations: one without any strict instruction about the residing time in the via-point (NoDwell) and the second with an imposed 500ms dwell time in the via-point (Dwell). When there is no instruction about the dwell time, the presence of an after effect to the movement correction became evident as the hand path deviation after the via-point that was opposed to the perturbation that was present before the via-point. However, comparable after effect is observed when there is a 500ms dwell time at the via-point. This supports the view that online corrections to force field perturbations within the movements could be preserved in the memory for at least 500ms of resting time. As such, the within sequence adjustments of reach representation highlighted without dwell time, which produced an after effect in less than 500ms, almost certainly shares most features with after-effects observed from trial to trial in standard adaptation experiments. In conclusion, our data further support that the fast time scales of motor adaptation are sufficiently fast to complement feedback and adapt an on-going movement.

Disclosures: P. Lefevre: None. **J. Mathew:** None. **F. Crevecoeur:** None.

Poster

229. Sensorimotor Learning I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 229.13/N29

Topic: E.04. Voluntary Movements

Title: Recreating the prism adaptation paradigm in an immersive virtual environment

Authors: *A. T. KAARIAINEN[1], R. E. JOHNSON[2], J. D. WILL[1];
[1]Electrical and Computer Engin., [2]Mechanical Engin. and Bioengineering, Valparaiso Univ., Valparaiso, IN

Abstract: Sensorimotor adaptation experiments are widely used to study the learning processes of the brain and to improve motor function for rehabilitation patients. One of the first adaptation experiments was the classical prism adaptation paradigm, which uses physical optics to introduce a visual perturbation and induce motor adaptation. Our research seeks to recreate the prism adaptation paradigm using a modern virtual reality head-mounted display (HMD), the HTC Vive Pro. We developed an application that allows us to measure sensorimotor adaptation in an immersive virtual reality environment and to explore the feasibility of using a consumer-grade HMD as a tool to study characteristics of motor adaptation.
The HMD is tracked by two base stations which allows the subject to move around and interact with a virtual 3D space the size of a room. We programmed a tracked object to act as a throwable ball whose position is aligned in the virtual world and in the physical world. The subjects threw the tracked object at a virtual target 8 ft away, and the object landed in a foam-padded area in the physical world. Two trackers were used: the first tracker was unperturbed and the second tracker had a -30° translation applied to it after being thrown. This perturbation was developed to mimic that of wearing the prism glasses in the classical experiment. We followed a procedure of a series of 20 tosses with unperturbed, 20 tosses perturbed, 20 tosses unperturbed, 20 tosses perturbed, 10 tosses unperturbed, 10 tosses perturbed, 10 tosses unperturbed, and 10 tosses perturbed. We then measured the subjects response to the introduced perturbation by recording the horizontal distance from the target on each throw.
We observed patterns of adaptation that in general are consistent with those we see outside of virtual environments. For example, even with no contextual cues, in immersive virtual reality, subjects adapted more quickly to familiar perturbations--a phenomenon often called savings. The HTC Vive Pro shows promise as a tool to study the various characteristics of motor adaptation, and the programming flexibility of a virtual environment enables a wide range of easily modifiable perturbations and scenarios.

Disclosures: **A.T. Kaariainen:** None. **R.E. Johnson:** None. **J.D. Will:** None.

Poster

229. Sensorimotor Learning I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 229.14/N30

Topic: E.04. Voluntary Movements

Support: NSF 1553895

Title: Examining the changes in the temporal stability of explicit and implicit learning due to normal aging

Authors: *W. ZHOU[1], R. D. NORTH[2], W. M. JOINER[1];
[1]Dept. of Neurobiology, Physiol. and Behavior, [2]Biomed. Engin., Univ. of California Davis, Davis, CA

Abstract: Normal aging is associated with declines in motor and cognitive abilities. In both cases this may involve deficits in memory and learning. Here, we utilized a well-known motor adaptation task (visuomotor rotation, VMR) to examine the temporal properties of two forms of learning (explicit or implicit) due to normal aging. We recruited young control subjects (18 to 22 years of age) and healthy elderly subjects (≥ 60 of age). All subjects made 10 cm point-to-point reaching arm movements using a cylindrical handle on a Wacom tablet while seated in front of an LCD monitor. Hand position was represented by a screen cursor (vision of the arm movement was hidden). At the start of each trial, a circle with numerical markers (spacing of 5.625º) centered at the start target was shown. Subjects were instructed to turn a rotation dial with their left hand to select a number on the display to indicate their aiming direction. Once the subject made a selection, the display was removed. Subjects were instructed to move the cursor from the start position to the target as quickly as possible. Following two blocks of baseline trials, each subject trained to perform movements to two targets located at 56.25º and 123.75º on the screen with a 45º or -45º visuomotor rotation of the visual feedback. Subjects only experienced one of the rotation directions, and the rotation direction was counterbalanced across subjects. After training, subjects completed a series of re-adaptation trials and retention probe trials for 6 randomly selected delay periods (0, 6, 10, 20, 50, and 90 seconds). We determined the explicit learning by the numerical selection of the intended trajectory direction prior to each movement and obtained implicit learning by subtracting this planned movement angle from the actual movement trajectory angle. These respective angles were converted to a ratio relative to the perturbation (45º or -45º). The young subjects showed almost complete retention of the explicit aiming, but an exponential decrease in the implicit learning component, consistent with previous work. However, the older subjects showed difficulty in learning the overall motor adaptation. The results of the older subjects also suggest that both the implicit and explicit learning stability was less than that demonstrated by the younger subjects. These results provide an initial assessment of the baseline measures necessary to distinguish the motor learning and memory deficits that accompany normal aging from impairments caused by brain disorders that progress with age (e.g., Alzheimer's disease).

Disclosures: W. Zhou: None. **R.D. North:** None. **W.M. Joiner:** None.

Poster

229. Sensorimotor Learning I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 229.15/N31

Topic: E.04. Voluntary Movements

Support: Wellcome Trust-DBT India Alliance Early Career Fellowship IA/E/14/1/501806
NICHD R01 HD075740

Title: Intermittent theta-burst magnetic stimulation over somatosensory cortex increases consolidation of motor learning

Authors: *N. KUMAR[1,2], T. M. MANNING[1], D. J. OSTRY[1,3];
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Abstract: There has been recent interest in the idea that motor learning does not occur in isolation but rather that motor learning involves changes to sensory systems and sensory networks in the brain. As an example, previous studies of sensorimotor adaptation have shown that motor learning is associated with a systematic change in the sensed position of the limbs. Consistent with this finding, neuroimaging studies have found learning-related changes in both sensory and motor areas of the brain. Recently we have shown that somatosensory cortex participates in the consolidation of motor memories developed through motor learning. Here we test the hypothesis that excitatory stimulation over somatosensory cortex following learning will increase the consolidation of motor memory. Participants perform a motor learning task involving force-field adaptation. Immediately following adaptation, we apply intermittent theta-burst transcranial magnetic stimulation (iTBS) to somatosensory cortex with the goal of enhancing motor memory consolidation. Subjects return to the laboratory 24 hours later to test for both memory retention and relearning by using a force-field perturbation that is applied in a direction opposite to that on the original training day. To date, subjects in the somatosensory group showed greater interference in learning an opposite perturbation than subjects in a sham-stimulation group, which indicates enhanced motor memory consolidation after stimulation. These results corroborate previous findings that somatosensory cortex is involved in the initial consolidation of motor memories developed during learning and show that the strength of these memories can be increased by magnetic stimulation.

Disclosures: **N. Kumar:** None. **T.M. Manning:** None. **D.J. Ostry:** None.

Poster

229. Sensorimotor Learning I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 229.16/N32

Topic: E.04. Voluntary Movements

Support: NSERC
Ontario Graduate Scholarship

Title: Aging, movement variability and motor adaptation

Authors: *L. LUSTIC, L. BROWN;
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Abstract: A controversial topic in motor learning literature is whether or not increased movement variability is beneficial or detrimental to motor adaptation. Counterintuitively, some research has shown that people who move more variably (in a task-relevant manner) are faster to learn a subsequent motor adaptation task. We know that movement variability increases with age. Does increasing age-related movement variability aid or hinder motor adaptation? We tested the hypothesis that increased age-related movement variability would result in faster learning using a mass adaptation task. Older and younger individuals completed a task in which they were asked to reach to targets appearing to the left or to the right of a fixed start location, as quickly and accurately as possible. They completed 100 baseline (no-mass) trials and then we applied a small weight to the participant's right arm during the adaptation block for another 100 trials. All participants completed 50 no-mass post-adaptation trials. We measured movement time, accuracy and spatial and temporal variability. We were interested in measuring our participants' inherent movement variability (during baseline trials) to determine the effect this variability had on the speed with which their reaching movements adapted to the applied weight. In our initial findings, older adults are displaying greater movement variability than our younger adults. Next, we will determine differences in group adaptation rates through measures of speed, accuracy and precision during practice. This research is important in determining whether or not certain aspects of aging may be hindering or helping motor adaptation, particularly in the presence of the current stigma around aging as a time of decline.

Disclosures: L. Lustic: None. **L. Brown:** None.

Poster

229. Sensorimotor Learning I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 229.17/N33

Topic: E.04. Voluntary Movements

Support: NSERC

Title: Multiple motor memories are formed for different control units linking the controlled point on a manipulated object and the target location

Authors: *M. R. MCGARITY-SHIPLEY[1], J. B. HEALD[3], J. N. INGRAM[3], J. P. GALLIVAN[1,2], D. M. WOLPERT[3], J. R. FLANAGAN[1];
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Abstract: Skilled manipulation requires forming and recalling memories of object dynamics, linking the force applied to an object to its resulting motion. Although it has been assumed that such memories are linked to objects, per se, we recently showed that people can form separate memories, for opposing dynamics, when these are linked to different locations, or 'control points', on an object [1]. In our previous study, participants moved a virtual horizontal bar with circles on the left and right, attached to the handle of a robotic device, straight ahead. In different trials, they were instructed to move either the left or right circle (control point) to a target located on the left or right, respectively. Participants successfully adapted to opposing force fields linked to these two contexts, even though the required movement was constant. In this previous study, both the controlled point and the target location changed between contexts. The aim of the current study was to assess whether one or both of these factors is critical for learning. The first experiment was similar to our previous study, except that the bar automatically rotated as it was moved forward such that the left and right control points, controlled in different contexts, moved to a common target. In the second experiment, the bar was aligned vertically with a single control point (circle) at the far end. Again, the bar rotated as it was moved forward such that the control point moved to a target located on either the left or right. We found that, in both experiments, participants learned opposing force fields applied in the two contexts. We conclude that separate memories of dynamics can be formed for different 'control units' involving a unique combination of control point and target.
[1] Heald JB, Ingram JN, Flanagan JR, Wolpert DM (2018) Multiple motor memories are learned to control different points on a tool. Nature Human Behaviour 2: 300-311.

Disclosures: M.R. McGarity-Shipley: None. **J.B. Heald:** None. **J.N. Ingram:** None. **J.P. Gallivan:** None. **D.M. Wolpert:** None. **J.R. Flanagan:** None.

Poster

229. Sensorimotor Learning I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 229.18/N34

Topic: E.04. Voluntary Movements

Support: NSF Grant 1342962
NSF Grant 1753915

Title: Somatosensory versus cerebellar contributions to complex motor skill learning: A theta burst stimulation study

Authors: *J. L. MIRDAMADI, H. J. BLOCK;
Dept. of Kinesiology and Program in Neurosci., Indiana Univ., Bloomington, IN

Abstract: Motor learning involves changes in behavior through practice and has been associated with plasticity in motor brain regions. It is well established that sensory information is important for motor control, yet its role in learning and associated plasticity has only recently been investigated. We previously demonstrated that complex motor skill learning is associated with enhanced proprioceptive function and changes in sensorimotor neurophysiology. Proprioceptive information is processed by the cerebellum and somatosensory cortex (S1), but the degree to which either structure contributes to proprioceptive improvements in motor skill learning is unknown. Here we used continuous theta burst stimulation (cTBS) after complex motor skill practice to determine the role of cerebellum and S1 in retention of proprioceptive changes and skill learning. 17 healthy young adults made visually-guided 2D reaching movements through an irregular-shaped track (20 cm x 20 cm horizontal workspace) as accurately as possible using a robotic manipulandum with their right hand. Subjects practiced movements over two consecutive days at a restricted speed range. Before and after practice, proprioception was measured using a passive two-alternative choice task to quantify bias (accuracy) and sensitivity (acuity). At the end of training on day 1 and 2, cTBS was delivered over either the right lateral cerebellum (CB), left S1, or with the coil tilted as a sham control. We compared proprioception function and motor skill learning (speed-accuracy tradeoff) at baseline to retention on day 3. Consistent with our previous behavioral experiment, the sham group (N = 6) appeared to demonstrate skill learning (40.2 ± 17.3%, mean increase in skill ± standard error), and improvements in proprioceptive sensitivity (8.4 ± 6.3 mm more sensitive) at retention. In contrast, the CB group (N = 6) on average showed no skill learning (0.22 ± 5.6%), with 4 subjects performing worse at retention. The S1 group (N=5) showed some evidence of skill learning on average (14.9 ± 2.3%). Both the CB and S1 groups had worsened proprioceptive bias at retention (4.9 ± 5.2 mm and 9.3 ± 10.5 mm more biased, respectively). However, sensitivity appeared to worsen only in the S1 group (6.5 ± 7.2 mm less sensitive). These preliminary findings provide some evidence that both the

cerebellum and S1 are important for retention of proprioceptive improvements related to skill learning. Consideration of regions outside of motor cortex, including those that contribute to proprioceptive processing, will be important for understanding the mechanisms mediating motor skill learning.

Disclosures: J.L. Mirdamadi: None. **H.J. Block:** None.

Poster

229. Sensorimotor Learning I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 229.19/N35

Topic: E.04. Voluntary Movements

Support: NSERC RGPIN-2017-04684
Canadian Foundation for Innovation

Title: Network interactions of frontoparietal regions during visuomotor adaptation

Authors: *D. J. GALE[1], C. ARESHENKOFF[1], J. Y. NASHED[1], D. STANDAGE[3], J. FLANAGAN[1,2], J. P. GALLIVAN[1,2];
[1]Ctr. for Neurosci. Studies, [2]Dept. of Psychology, Queen's Univ., Kingston, ON, Canada; [3]Sch. of Psychology, Univ. of Birmingham, Birmingham, United Kingdom

Abstract: Previous studies have shown that sensorimotor learning is associated with activity changes in motor-related cortical brain areas, and is thought to reflect a learning-related reconfiguration of the motor system. To date, such changes in the motor system have largely been studied by contrasting brain activity at discrete time periods (e.g., before and after learning), and/or coarsely in the context of whole-brain networks. It remains unclear how learning-related changes evolve within the constituent areas of the cortical motor system during the entire time course of learning. Here, we used functional magnetic resonance imaging (fMRI) to explore time-evolving activity of sensorimotor-related regions within a frontoparietal network during visuomotor adaptation. Subjects underwent two identical fMRI sessions, in which scans were collected while subjects adapted, and subsequently de-adapted, to an instantaneous 45-degree rotation applied to the cursor, following baseline trials, during an 8-target center-out movement task. In addition, resting state scans were also collected before and after adaptation, and following de-adaptation. A sensorimotor network-of-interest was constructed using frontoparietal areas from a fine-scale parcellation scheme (Schaefer et al, 2018). Broadly, we hypothesized that the course of adaptation would be accompanied by time-varying changes in activity among network regions, therefore reflecting learning-related modulation of a dynamic network state. Testing this hypothesis using functional connectivity, we show that network interactions vary as a function of learning, and which differ from interactions observed at

baseline. Further, we demonstrate how properties of network structure, through time, relate to aspects of learning behaviour across subjects. Altogether, our findings demonstrate how the cortical motor system reconfigures itself over the time course of learning and how these network-level changes are linked to individual differences in behaviour.

Disclosures: D.J. Gale: None. **C. Areshenkoff:** None. **J.P. Gallivan:** None. **D. Standage:** None. **J.Y. Nashed:** None. **J. Flanagan:** None.

Poster

229. Sensorimotor Learning I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 229.20/N36

Topic: E.04. Voluntary Movements

Support: NSERC RGPIN-2017-04684

Title: Network-level interactions during sensorimotor adaptation learning and generalization

Authors: *C. N. ARESHENKOFF[1], A. J. DE BROUWER[2], J. NASHED[1], D. GALE[1], J. P. GALLIVAN[1];
[1]Ctr. for Neurosci. Studies, Queens Univ., Kingston, ON, Canada; [2]Univ. of British Columbia, Vancouver, BC, Canada

Abstract: An important feature of the success of many well-learned skills is that they generalize to new contexts. As such, studies on the generalization of learning have provided a unique window onto the basic nature of motor learning, and have been shown to have key implications for improving motor rehabilitation and training. One major paradigm in which motor generalization has been studied is through intermanual transfer, which examines how adaptation generalizes from the trained hand to the untrained hand. Given the contralateral organization of the motor system, this form of generalization is thought to rely on significant interhemispheric coordination, but supporting neural evidence remains sparse.
We study covariance networks comprising the cerebellar hemispheres, striatum, and cortical motor regions obtained by functional magnetic resonance imaging (fMRI) during adaptation to a visuomotor rotation and its subsequent generalization to the untrained hand. In separate blocks, right handed subjects moved a cursor towards a target using either their left or right hands, both in the presence and absence of a visuomotor rotation, in which the correspondence between cursor and hand was rotated by 45 degrees. By leveraging the natural geometry of the space of covariance matrices, we isolated hand and rotation specific covariance profiles for each subject, and found that both response hand and the presence or absence of rotation could be decoded in unseen subjects with high accuracy.
Left handed performance was associated with greater BOLD signal correlation between

homologous regions in the left and right hemispheres overall, consistent with research demonstrating greater ipsilateral motor activation for left-handed movements in right-handed subjects. Moreover, the effect of rotation was markedly different for right- vs. left-handed responses, possibly reflecting intrinsic differences in networks underlying right vs. left-handed responding. We examine the relationship between network architecture and behavior during adaptation, as well as during the transfer of a learned rotation to a new hand.

Disclosures: C.N. Areshenkoff: None. **A.J. de Brouwer:** None. **J. Nashed:** None. **D. Gale:** None. **J.P. Gallivan:** None.

Poster

229. Sensorimotor Learning I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 229.21/N37

Topic: E.04. Voluntary Movements

Title: Using a chopping task to compare expert and competent motor memory

Authors: *A. H. NEPOTIUK, L. E. BROWN;
Trent Univ., Peterborough, ON, Canada

Abstract: Typically task interference is studied using reaching adaptation tasks (visuomotor rotation and/or force-field learning). Participants' ability to adapt reaching to the imposed perturbation is studied. The pattern of data induced by the perturbation is used to make inferences about the nature and neural correlates of our learning and memory for reaching perturbations, specifically, and motor performance in general. We wanted to see if it is possible to demonstrate this same interference pattern using a novel vegetable-chopping task, where we can easily recreate natural performance settings using a task for which we can easily identify competent and expert performers. Here we begin to test the idea that at least for knife skills, expertise is defined both by the range of skills learned (the ability to work with different materials at different speeds using either the same or different instruments) and the refinement of performance for each individual skill. We hypothesize that the motor memories of experts are structured differently from those of competents and that experts' memories for tasks are organized in a way that allows them to move from one task to another without experiencing interference. We tested this hypothesis by exposing experts and competents to an interference paradigm, and predicted that experts would be less vulnerable to interference than competents. Trained chefs and competent home cooks performed a chopping task in which they were asked to chop a sweet potato into 5 mm-wide slices, matching the beat of a metronome (120 bpm). Following this initial block, participants were exposed to an altered frequency (100 bpm or 140 bpm) interference condition. Participants then performed trials of the original task again. Interference was inferred if the second performance of the original task was impaired, compared

to initial performance. As predicted, we found interference in competents, but not experts. These results support the idea that experts' motor memories are stored differently than competents', such that experts' task-specific memories are protected against interference. We will discuss these results with respect to different models of motor memory.

Disclosures: A.H. Nepotiuk: None. **L.E. Brown:** None.

Poster

229. Sensorimotor Learning I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 229.22/N38

Topic: E.04. Voluntary Movements

Support: NSF Grant 1553895

Title: Sensory dissociation reveals postural influences on motor adaptation

Authors: *J. J. FITZGERALD[1], W. ZHOU[2], S. M. CHASE[3], W. M. JOINER[2];
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Abstract: It has been well documented that adaptation to an external force-field applied during a reaching task is critically dependent on the motion state of the arm. However, even prior to the introduction of novel dynamics, these kinematic variables can alter the forces exerted during movement. We examined the influence of arm orientation and the dissociation of visual feedback on (1) the force patterns elicited before adaptation to a velocity dependent force-field, (2) the effects of these baseline forces on the time course and asymptotic levels of adaptation, and (3) the magnitude of transfer of adaptation to a novel workspace. We trained two groups of right-handed subjects (N = 16 in both groups) to make point to point reaching movements towards the body with the right arm using a robotic manipulandum. Subjects were trained in one of two workspaces: Ipsilateral (9 cm right of midline) or Contralateral (9 cm left of midline). In these workspaces subjects were trained in one of two force directions: towards or away from the midline. Error clamp force profiles were probed in both workspaces, with and without visual feedback dissociation (displaying the cursor in the workspace opposite the hand), before, throughout and after 120 training trials. After training, an additional delineation of the dissociation cases was made. Visual Transfer: arm remained in the trained workspace while the cursor was shown in the other. Postural Transfer: the cursor remained in the trained workspace while the arm moved in the other. We found that moving in the contralateral workspace, or adding a visual dissociation, significantly increased forces during baseline performance ($p < .05$). The combination of both conditions resulted in force greater than either individual case ($p < .05$).

In all cases, forces were directed towards the midline. A gain space model with significant position, velocity, and acceleration dependent components accurately modeled these baseline force profiles ($p < .05$). The position component of baseline forces was found sensitive to arm orientation, but not the presence of a visual dissociation ($p < .05$). The velocity component was sensitive to the presence of a visual dissociation, but not arm orientation ($p < .05$). The acceleration component was sensitive to both arm orientation and the presence of a visual dissociation ($p < .05$). The position component present during baseline persisted through force-field training. These results suggest that biomechanical (via the arm orientation) and cognitive processes (via the visual dissociation) affect the force patterns produced during reaching tasks and suggest biomechanical effects cannot be readily overcome by cognitive processes.

Disclosures: J.J. Fitzgerald: None. **S.M. Chase:** None. **W.M. Joiner:** None. **W. Zhou:** None.

Poster

229. Sensorimotor Learning I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 229.23/N39

Topic: E.04. Voluntary Movements

Support: NSF Grant 1553895

Title: The role of an explicit strategy in the savings of adaptation to novel dynamics

Authors: *R. D. NORTH, W. ZHOU, W. M. JOINER;
Dept. of Neurobiology, Physiol. and Behavior, Univ. of California Davis, Davis, CA

Abstract: Reaching movements rapidly adapt in response to external perturbations (e.g., manipulations of movement dynamics or visual feedback). When exposed to the same type of perturbation, human subjects demonstrate a faster learning rate compared to the timecourse of initial training (e.g., savings). Recent evidence suggests that explicit learning mechanisms are largely responsible for savings in response to alterations of visual feedback. However, the role of explicit learning during the savings of adaptation to novel movement dynamics (e.g., force-field adaptation) is less clear. Here, we directly examined the effects (and extent) of explicit visual feedback on savings in a force-field learning task. We tested two subject groups (implicit learning and explicit feedback). Similar to previous studies, subjects in the implicit learning group experienced the novel movement dynamics (a velocity-dependent force-field in which the lateral perturbation scaled with velocity). Subjects in the explicit feedback group moved in force channels and we measured the lateral force exerted by the subject. On each trial we provided visual feedback of this temporal force profile as well as the required force pattern based on the movement distance and velocity. Thus, subjects were provided explicit feedback on the extent the motor output matched the required velocity-dependent force profile. Both subjects

experienced 120 trials of training in these respective paradigms. After training, both subject groups experienced a washout block of 80 consecutive null trials (with no visual feedback of the force or perturbation) which was then followed by a second training block to assess savings. Results show that the initial learning rate and adaptation rate during retraining for the explicit feedback group was faster in comparison to the implicit learning group. Thus, the results suggest that an explicit strategy can contribute and increase the savings observed for adaptation to novel movement dynamics. This type of paradigm may allow for the dissociation of implicit and explicit learning mechanisms in other aspects motor adaptation behaviors (e.g., generalization and the temporal stability of learning).
Theme and Topic: Reaching control - Motor learning - Human
Keyword: Motor Learning

Disclosures: R.D. North: None. **W. Zhou:** None. **W.M. Joiner:** None.

Poster

229. Sensorimotor Learning I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 229.24/N40

Topic: E.04. Voluntary Movements

Support: NIH Grant 1R15AG059095-01

Title: Acquisition, short-term and long-term retention of sensorimotor adaptation in healthy aging and early Alzheimer's disease

Authors: *B. E. DUROCHER[1], A. T. WATRAL[1], L. RAJESHKUMAR[1], K. M. TREWARTHA[1,2];
[1]Cognitive and Learning Sci., [2]Kinesiology & Integrative Physiol., Michigan Technological Univ., Houghton, MI

Abstract: When learning a new motor skill, declarative memory processes allow us to make rapid improvements in performance initially, and likely allow us to retain recently acquired motor skills over time. Amnestic mild cognitive impairment (MCI) and Alzheimer’s disease (AD) are associated with significant impairments in declarative memory resources both for learning, and retention of information over short and long delays. Here, we investigate whether the early stages of motor learning are affected by MCI and early AD, and whether those patients exhibit additional impairments in short-term (i.e., within session) and long-term (after a 24-hour delay) retention of a newly acquired motor skill. For this project we recruited participants diagnosed with MCI and the early stages of AD, as well as control groups of cognitively healthy older and young adults to perform a force-field adaptation task using a robotic manipulandum (KINARM, B-kin Technologies). Participants attempt to reach towards visual targets, while the

robot applies a velocity-dependent force perpendicular to the direction of the target. While this load initially perturbs hand movement, participants gradually adapt by producing forces that counteract the load. On Day 1, participants adapt to the force-field and then perform a final block of error-clamp trials to assess short-term retention of motor adaptation. On Day 2, participants return 24 hours later to perform the same motor learning task again to assess long-term retention of the motor behavior. All older participants also perform a standard neuropsychological assessment of cognitive function to quantify the severity of the cognitive impairments in the MCI and AD participants. We compare the initial speed of acquisition in the first session between groups to establish the effect of MCI and AD on early stages of motor learning. We also compare short-term retention of the newly acquired motor behavior during an error-clamp phase following initial adaptation. Finally, we examine the impact of MCI and AD on the long-term retention of motor learning by assessing group differences in savings when participants perform the force-field adaptation task 24 hours later. The overarching goal of this work is to determine whether acquisition, and short- and long-term retention measures in motor learning can distinguish between MCI, AD, and healthy aging to potentially supplement existing neuropsychological measures for diagnosing AD.

Disclosures: B.E. Durocher: None. **A.T. Watral:** None. **L. Rajeshkumar:** None. **K.M. Trewartha:** None.

Poster

229. Sensorimotor Learning I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 229.25/N41

Topic: E.04. Voluntary Movements

Support: Athletic and Human Performance Research Center (AHPRC), Marquette University

Title: Does visual capture explain adaptation to simultaneous visuomotor and manuomotor perturbations?

Authors: *D. PETERS[1], D. LANTAGNE[1], J. HOELZLE[2], C. S. SMITH[3], D. G. THOMAS[4], L. A. MROTEK[1], R. A. SCHEIDT[1,5,6];
[1]Biomed. Engin., Marquette Univ. and Med. Col. of Wisconsin, Milwaukee, WI; [2]Psychology, [3]Marquette Univ. Med. Clin., Marquette Univ., Milwaukee, WI; [4]Pediatrics, Med. Col. Of Wisconsin, Milwaukee, WI; [5]Feinberg Sch. of Med., Northwestern Univ., Chicago, IL; [6]Div. of Civil, Mechanical and Manufacturing Innovation, Natl. Sci. Fndn., Alexandria, VA

Abstract: Visual capture refers to the phenomenon where visual stimuli can dominate multisensory perception. Experimental studies differ in the extent to which visual feedback

dominates sensorimotor adaptation. Visual capture was observed when visual and proprioceptive cues varied unpredictably from one movement to the next (Judkins and Scheidt, J. Neurophysiol., 2014). Visual capture was not observed when visual and proprioceptive perturbations were deterministic, allowing subjects to adapt to a visuomotor rotation and altered limb dynamics in parallel (Krakauer et al., Nature Neurosci., 1999). We tested whether visual capture occurs when proprioceptive uncertainty is great by challenging subjects to perform goal-directed reaching with a predictable visuomotor rotation and unpredictable force perturbations. 6 subjects grasped the handle of a two-joint robot and performed 200 consecutive goal-directed, out-and-back reaches in the horizontal plane against spring-like force field perturbations that changed in strength on each trial. Direct view of the arm was blocked. Subjects were given faithful visual feedback of hand location via a vertically oriented monitor during the first 30 trials (baseline block). Then a 30-degree visuomotor rotation was implemented for 140 trials (rotation block). The visuomotor rotation was removed for the last 30 reaches (washout). Kinematic performance was quantified by direction error at the moment of peak velocity and Euclidian error at the end of the reach (target capture error). We compared the final 10 trials of baseline to the first 10 trials of the rotation block, the final 10 trials of the rotation block, and the first 10 trials of the washout block. Time series analysis examined how participants used sensorimotor memories to adapt to the unpredictable force perturbations while the visual motor rotation was present. Direction error increased for initial trials of the rotation block ($t(5)=5.36$, $p<0.004$), decreased as the participants adapted to the visuomotor rotation ($t(5)=5.35$, $p<0.004$) and exhibited washout when the rotation was removed. Thus, the participants adapted to the visuomotor rotation. Time series analysis found that target capture error on any given trial was influenced by force perturbations and performance errors on the previous trial, consistent with previous models of sensorimotor adaptation. Thus, participants adapted to force perturbations and to the visuomotor rotation in parallel. Visual capture is not predicated by the elevated levels of proprioceptive uncertainty induced by unpredictable manuomotor perturbations during simultaneous adaptation to a visuomotor rotation.

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Poster

229. Sensorimotor Learning I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 229.26/N42

Topic: E.04. Voluntary Movements

Support: R01 AG041878

Title: Motor performance error contributes more strongly than sensory prediction error to short-term implicit motor adaptation

Authors: *H. OH[1], M. A. SMITH[2];
[2]Sch. Engin., [1]Harvard Univ., Cambridge, MA

Abstract: Sensory prediction error (SPE) and motor performance error (MPE) are two distinct error signals that have long been hypothesized to play different roles in motor learning. However, little is known about the difference between how these two error signals contribute to motor learning. Thus, here we created an experimental paradigm to dissociate the effects of SPE and MPE.
We accomplished this by combining cursor perturbations and target perturbations. The experiment combined three conditions: visuomotor cursor rotation trials without target jump (VMR-only), target jump trials without visuomotor cursor rotation (TJ-only), and trials where visuomotor cursor rotation and target jump were both present (VMR-TJ). The three trial conditions were randomly interleaved, and the amplitudes of visuomotor cursor rotations and target jumps, when they occurred, were randomized, taking the values of ±4, ±2, or 0°. The idea here is that VMR-only trials would produce both SPE (because the cursor motion would be different than predicted) and MPE (because the perturbed cursor motion would affect the ability of the cursor to reach the target). However, TJ-only trials would produce MPE (because the jumped target would affect the ability of the cursor to reach it), but not SPE (because the jumped target would not affect cursor motion). In the third condition, VMR-TJ trials, we jumped the target in the same direction and amplitude as the cursor rotation, so that MPE would be unaffected because the effects of VMR and TJ would cancel, however, SPE would be present due to the perturbed cursor motion. We then computed the single-trial adaptation associated with VMR-only, TJ-only and VMR-TJ conditions at each perturbation amplitude (±4, ±2°). We found the single-trial adaptation rate of 0.17 when SPE and MPE were both induced (VMR-only trials). When only SPE was induced, the amplitude of adaptation was significantly reduced (nominally, by >70%). When only MPE was induced, the amplitude of adaptation was not significantly reduced (nominally, by <30%). Previous work with a similar randomized small perturbation paradigm found essentially zero explicit learning. If applicable here, the current findings would indicate that MPE drives short-term implicit adaptation more strongly than does SPE.

Disclosures: H. Oh: None. **M.A. Smith:** None.

Poster

229. Sensorimotor Learning I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 229.27/N43

Topic: E.04. Voluntary Movements

Title: The effect of visual feedback latencies on the adaptation and generalization of motor adaptation

Authors: *G. ABRAHAM[1], T. RANJAN[2], M. A. SMITH[3];
[2]Sch. of Engin. and Applied Sci., [3]Sch. Engin., [1]Harvard Univ., Cambridge, MA

Abstract: The human motor system displays sensorimotor loop delays of 150 ms or more for visual information. These delays can be increased tremendously in motor tasks where feedback of action consequences is delayed (e.g. bowling and throwing darts). These delays are also increased with human-computer interfaces (HCIs), such as those used in many motor learning experiments, where excess latencies of 50 to 100+ms are common. Studies of prism adaptation (Kitazawa, 1995) and visuomotor rotation (Taylor et al, 2016) have reported reduced motor learning when visual latencies were experimentally increased, but the baseline HCI latencies in these studies were unfortunately not measured.

Here we studied the effect of multiple visual feedback latencies on both implicit and explicit motor adaptation in a visuomotor rotation (VMR) paradigm, and on the generalization of implicit and explicit adaptation to untrained movement directions. We began by creating an experimental setup with a measured HCI latency of only 20-25 ms, and studied the effects of experimentally adding 0, 60, and 275 ms delays to visual feedback in separate blocks of trials. The 0ms delay group showed 29°/30° of asymptotic adaptation by the end of training, comprised of 8° of explicit learning and 21° of implicit learning. The 60 and 275 ms delay groups displayed unchanged overall adaptation by the end of training, however implicit learning was reduced (by 34 and 52%, respectively, $p=10^{-2}$ and $p=10^{-4}$) whereas explicit learning was increased (by 73% and 137%).

Overall, we found that the presence of additional visual feedback delays lead to adaptation that was less local than the baseline condition. In particular, we found that explicit learning (which increased with higher visual feedback delays) generalized broadly and indiscriminately across all movement directions, and the amplitude of generalization for the explicit learning proportional to the asymptotic level of explicit learning during the training period. In contrast, implicit learning (which decreased with higher visual feedback delays) generalized with a combination of global and local components. The local component had with a width (sigma) of ~30° and decreased in proportion to the asymptotic level of implicit learning during the training period, however the global component (which was smaller) was essentially identical for all 3 delay conditions. These results suggest that relatively small visual feedback latencies can dramatically alter the shape of the generalization function that arises from motor adaptation and the composition of motor adaptation in terms of explicit and implicit contributions.

Disclosures: **G. Abraham:** None. **T. Ranjan:** None. **M.A. Smith:** None.

Poster

229. Sensorimotor Learning I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 229.28/N44

Topic: E.04. Voluntary Movements

Title: Can we unlearn how to ride the bicycle?

Authors: J. MAGNARD, T. MACAULAY, E. SCHROEDER, J. GORDON, ***N. SCHWEIGHOFER**;
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Abstract: Riding a bicycle is the classic example of a motor skill in which the rules governing successful performance are learned implicitly and, once learned, the skill is highly durable. A key rule for maintaining balance on a bicycle is that the rider must use the handlebars to turn the front wheel toward the side of a tilt. In this study, we investigated whether experienced bicycle riders could learn to ride a modified bicycle with reversed handlebar-to-wheel coupling so that when the handlebar is turned right the wheel turns left and vice versa. This requires riders to learn a new rule - to turn the handlebars away from the side of a tilt. We also investigated whether learning to ride the reversed bicycle would affect their ability to ride a normal bicycle. *Methods*. 14 healthy adults (mean age: 25.7 years) performed 8 training sessions on the reversed bicycle (10 minutes each) on separate days. Their goal was to ride the reversed bicycle for 20 meters. Riding distance was recorded at the beginning (*pre*-) and at the end (*post*-) of each session for five trials. At the beginning of the 5^{th} session and during a final evaluation one day following the last session, subjects were asked to ride 20 meters with the normal bicycle. The number of attempts necessary to ride 20 meters with the normal bicycle was used to quantify the interference caused by learning the reversed bicycle. *Results*. All subjects improved the distance ridden with the reversed bicycle across sessions (*e.g*., *pre*-training session #1: 1.5 ± 0.3 (mean ± SD) meters *vs*. *post*-training session #8: 15.9 ± 6.3 meters). However, three subjects were not able to ride 20 meters with the reversed bicycle after 8 training sessions. During the learning process, subjects became unable to ride the normal bicycle: 3.6 ± 3.2 trials (min: 1, max: 12) and 11.7 ± 10.5 trials (min: 2, max: 35) were required to ride 20 meters with the normal bicycle in the 5^{th} session evaluation and the final evaluation, respectively. However, three distinct patterns were observed: Both “poor” and “expert” learners could ride the normal bicycle only after a few trials. In contrast, “intermediate” leaners required a large number of trials to ride the normal bicycle again. *Discussion*. Our results show that most subjects can learn the reversed bicycle rule. However, learning to ride the reversed bicycle interferes with performance on the normal bicycle, but only during the acquisition process. With sufficient practice with the reversed bicycle, interferences become minimal, indicating that both reversed and normal bicycle rules

can be stored as separate memories. Thus, learning to ride the reversed bicycle requires subjects to temporarily "unlearn" how to ride a normal bicycle.

Disclosures: **N. Schweighofer:** None. **J. Magnard:** None. **T. Macaulay:** None. **J. Gordon:** None. **E. Schroeder:** None.

Poster

229. Sensorimotor Learning I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 229.29/N45

Topic: E.04. Voluntary Movements

Title: On the encoding capacity of human motor adaptation

Authors: *S.-H. YEO[1], S. KIM[2], J. KWON[2], J. KIM[1], F. PARK[2];
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Abstract: Primitive-based models of motor learning suggest that motor skill is internally represented as response characteristics of motor primitives, each of which is activated by a specific state of the movement. Based on this idea, we consider motor learning as an information encoding procedure, that is, a procedure of encoding a motor skill into primitives. Since the encoding capacity, the number of primitives involved, is limited by the range of the state covered by the movement, this leads to a rather counter-intuitive prediction that faster movements can encode more information, i.e. more complicated motor skills. In addition, since the performance of faster movements declines due to signal-dependent noises, we hypothesize that the net performance of motor learning is determined by reconciling the trade-off between the effect of encoding capacity (faster the better) and that of signal-dependent noise (slower the better). To verify the hypothesis, two experiments were conducted. The first experiment was designed to validate the existence of such trade-off. While holding the handle of a planar robotic arm (vBOT), subjects made circular movements against a perturbing angle-dependent radial force field. The experiment was conducted in two-by-two conditions: 1) faster vs slower speed and 2) higher vs lower skill complexity, defined as the number of switches between inward and outward radial force. The analyses were focused on the effect of the movement speed on the adaptation performance for different complexity conditions. The result showed the adaptation was significantly better at a slower speed in the lower complexity condition, but significantly better at a higher speed in the higher complexity condition, which confirms the existence of the trade-off. The second experiment was aimed to check if the observed effect is not from simple muscle co-contraction strategies. The same handle-rotating experiment was done in two conditions: 1) the "normal" condition where the radial force field is fixed over the trial and 2) the "asynchronous" condition where the force field slowly rotates during the trial, causing a conflict in encoding, but

preserving the overall characteristics of the force field. During the experiment, EMG of four upper arm muscles were also measured to check the co-contraction level. The result showed that the adaptation performance was significantly better in the normal condition, with a significantly lower level of muscle co-contraction. Taken together, these results suggest that the encoding capacity is a genuine limiting factor of motor adaptation and its interaction with signal-dependent error determines the adaptation performance.

Disclosures: **S. Yeo:** None. **S. Kim:** None. **J. Kwon:** None. **J. Kim:** None. **F. Park:** None.

Poster

230. Cells, Circuits, and Motor Patterns

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 230.01/N46

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NS066587
NS070583

Title: The functional significance of a tonic current induced during one type of motor activity may not become apparent unless there is a subsequent task switch

Authors: *Y. WANG, K. R. WEISS, E. C. CROPPER;
Neurosci., Icahn Sch. of Med. at Mount Sinai Hosp., New York, NY

Abstract: We study a multi-tasking network-the feeding circuit in *Aplysia*. This circuit generates egestive and ingestive motor programs, which differ in the phasing of activity in the B8 radula closer motor neurons. When B8 fires at a high frequency during radula protraction food is pushed out, i.e., egested. When B8 fires at a high frequency during retraction food is pulled in, i.e., ingested. Egestive motor programs can be triggered *in vitro* by stimulating an input to the feeding CPG, the esophageal nerve (EN). If cycles of motor activity are triggered with a relatively short intercycle interval repetition priming is observed. Activity that is initially intermediate is reconfigured and becomes progressively more and more egestive. This is manifested as a progressive increase in the B8 firing frequency during protraction. In principle this increase could be mediated by a progressive alteration in B8 synaptic input and/or by changes in B8 excitability. To determine which is the case we triggered motor programs by stimulating the EN and recorded from one B8 using current clamp and a second B8 using voltage clamp. As activity became egestive there was an increase in the peak amplitude of the inward synaptic current recorded during protraction. Additionally, there was a progressive increase in the magnitude of a persistent, tonic current. Surprisingly the tonic current was outward rather than inward. The outward current was, however, small when compared to the inward synaptic current recorded after priming. Consequently, the total amount of inward current induced during

protraction did in fact increase as priming occurred. Since induction of the outward current would not contribute to egestive priming an obvious question is, what is its function? We hypothesized that consequences of its induction might become apparent during a task switch -in particular a switch to ingestive activity. A previous study demonstrated that in this situation a switch cost is observed. Namely, after egestive priming stimulation of the ingestive command-like neuron CBI-2 triggers egestive activity. We hypothesized that the persistent outward current that develops during egestive priming might contribute to the retraction phase decrease in B8 activity observed during this task switch. To be able to manipulate the current using dynamic clamp techniques we determined its I-V characteristics. We then artificially introduced this current and found that there was a significant decrease in B8 excitability. This suggests that we have identified a persistent current induced during egestive priming whose function only becomes apparent when there is an attempt to task switch.

Disclosures: Y. Wang: None. **K.R. Weiss:** None. **E.C. Cropper:** None.

Poster

230. Cells, Circuits, and Motor Patterns

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 230.02/O1

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NS066587
NS070583

Title: The complement of projection neurons that triggers motor activity can determine whether a persistent state is created

Authors: *C. G. EVANS[1], M. A. BARRY[1], M. H. PERKINS[1], J. JING[1,2], K. R. WEISS[1], E. C. CROPPER[1];
[1]Icahn Sch. of Med. At Mount Sinai, New York, NY; [2]Nanjing Univ., Nanjing, China

Abstract: In *Aplysia*, a population of about 13 projection neurons in the cerebral ganglion (cerebral-buccal interneurons or CBIs) project to the buccal ganglia which innervate the buccal mass musculature (Wu et al. 2014). Individual CBIs have been shown to exhibit unique functions, from initiating motor output from the buccal ganglia to altering specific motor program parameters, e.g. motor output from the buccal ganglia can be ingestive, egestive or, have intermediate characteristics depending on the complement of projection neurons that are active. CBI-2, the most intensively studied projection neuron, reliably drives motor programs and is activated by food. When CBI-2 is repeatedly stimulated with a relatively short inter-stimulus interval (ISI), repetition priming is observed. That is, buccal motor programs are initially intermediate, progressively becoming fully ingestive over time, and a persistent

ingestive “state” is established. The ingestive “state”, slowly returns to an intermediate “state” if the CBI-2 ISI is sequentially increased (Proekt et al. 2004; Friedman et al. 2009). We now report that in some preparations, repetition priming does not occur and, motor programs remain intermediate when CBI-2 is repeatedly stimulated with a short ISI. We ask whether an ingestive configuration and a persistent ingestive state can be produced in non-priming preparations via co-stimulation of a second CBI. We selected CBI-3 since it is also activated by food and it can convert egestive activity to ingestive (Rosen et al. 1991; Morgan et al. 2002). When CBI-3 and CBI-2 were co-stimulated in non-priming preparations, motor programs did become ingestive. Interestingly, this occurred immediately, i.e., repetition priming was not necessary. To determine whether effects of CBI-3 co-activation were persistent, we conducted experiments in which CBI co-activation was followed by a return to motor program initiation using only CBI-2. Motor programs immediately became intermediate showing that a persistent state had not been established. This suggested that with CBI-2/CB-3 co-activation, it would be possible to rapidly return to an intermediate configuration. We confirmed that this is the case. Our data suggest that the neural circuit of the *Aplysia* feeding system can generate fully ingestive programs through either slow dynamic change of the network state by activation of a single command neuron, or faster dynamic changes through combined activation of multiple projection interneurons. The faster dynamic network change may facilitate task switching.

Disclosures: C.G. Evans: None. **M.A. Barry:** None. **M.H. Perkins:** None. **J. Jing:** None. **K.R. Weiss:** None. **E.C. Cropper:** None.

Poster

230. Cells, Circuits, and Motor Patterns

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 230.03/O2

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NS101356

Title: Population-level analysis of motor pattern initiation, selection, and termination in *Aplysia*

Authors: *S. C. PATWARDHAN[1], R. M. COSTA[1], R. HOMMA[1], D. A. BAXTER[2], J. H. BYRNE[1];
[1]Neurobio. and Anat., The Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX; [2]Engin. & Med. (EnMed), Texas A & M Hlth. Sci. Ctr. - Houston, Houston, TX

Abstract: Rhythmic behaviors are often mediated by specialized circuits termed central pattern generators (CPGs). Substantial progress has been made toward understanding the cellular and synaptic processes that underlie the genesis of rhythmic neural activity. However, broader aspects of CPG function, such as tracking and characterizing the neural state of the CPG as it

initiates, selects, and terminates patterned neural activity, are not well understood. With the advent of large-scale recordings of neuronal activity, it is possible to analyze the state of large complex circuits in increasing detail. Here, we describe methods for examining the extent to which both single-neuron and population-level activities can predict the initiation, selection, and termination of specific patterns of neural activity. Using voltage-sensitive dye imaging, we simultaneously monitored activity in ~100 cells in the buccal ganglia of *Aplysia*, which contain a CPG that mediates rhythmic feeding movements. This CPG generates buccal motor patterns (BMPs) corresponding to several types of feeding behaviors, such as ingestion (iBMPs) and rejection (rBMPs). We monitored and classified spontaneously generated BMPs using simultaneous recordings from buccal nerves. We used principal component analysis (PCA) to represent the activities of the ~100 cells in a low-dimensional space, and linear discriminant analysis (LDA) to find points in time at which neurons become predictive (Briggman et al., 2005). Preliminary results indicate that three principal components (PCs) are sufficient to capture distinguishing features of iBMPs and rBMPs. To examine the possibility that these features might be influenced by differences in the duration of the different pattern types (Nargeot et al., 2002), we normalized the timescale and repeated the analysis. We found that iBMPs and rBMPs are distinguishable (pattern selection) even when the patterns are compared on a normalized timescale. Lastly, we ranked neurons by how well they predicted pattern type, based on their weights across the first three PCs and the weights of the PCs across predictive linear discriminant functions. We found that the neurons that best predicted pattern type on the raw timescale also were the best predictors on the normalized timescale. These results outline a generalizable approach that will be useful for making population-level predictions regarding the initiation, selection, and termination of patterned activity in neural circuits.

Disclosures: **S.C. Patwardhan:** None. **R.M. Costa:** None. **R. Homma:** None. **D.A. Baxter:** None. **J.H. Byrne:** None.

Poster

230. Cells, Circuits, and Motor Patterns

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 230.04/O3

Topic: E.07. Rhythmic Motor Pattern Generation

Support: R35-NS097343
5T32-NS007292-31

Title: Characterization and comparison of two coupled CPGs as a function of temperature

Authors: ***D. J. POWELL**[1], S. A. HADDAD[2], S. GORUR-SHANDILYA[1], M. RUE[1], E. E. MARDER[3];

[1]Neurosci., Brandeis Univ., Waltham, MA; [2]Max Planck Inst. for Brain Res., Frankfurt, Germany; [3]Volen Ctr. and Biol. Dept., Volen Ctr., Waltham, MA

Abstract: Nearly all biological processes are affected by temperature. It is therefore remarkable that poikilotherms and their nervous systems can maintain activity across a wide range of temperatures. In the crab *C. borealis*, there are two central pattern generators (CPGs), the fast pyloric (filtering, ~1Hz cycle frequency) and slower gastric mill (chewing, ~0.1 Hz cycle frequency) sub-circuits within the stomatogastric nervous system. Pyloric neurons have been shown to maintain stable, rhythmic activity between 7 and 23°C (Tang et al. 2010). We asked whether the gastric mill CPG can maintain activity across a similar temperature range. Here we evaluated both spontaneously generated and electrically stimulated (evoked) gastric mill rhythms (GMRs) and found that rhythms can be maintained between 7 and 21°C. In these experiments, GMRs were evoked between 7 and 21°C, however spontaneous rhythms were observed at temperatures as high as 31°C. Although spontaneous and evoked gastric mill rhythms differ from one another in terms of neuron participation and phase relationships of gastric mill neurons, they retain a variety of similarities. Both spontaneous and evoked GMR frequencies increased with temperature, a phenomenon previously documented in the pyloric rhythm. Across this temperature range both the pyloric and gastric mill rhythm have similar apparent Q10s. Certain versions of GMRs are known to have an integer number of pyloric cycles per gastric mill cycle, termed integer coupling (Nadim et al. 1998; Bartos et al. 1999). We found that both spontaneous and evoked GMRs maintain integer coupling at baseline temperatures (11°C). Interestingly, with evoked gastric mill rhythms, integer coupling did not significantly vary with temperature ($p = 0.88$, $N = 8$), although the phase coherence between PD and LG neurons decreased with increasing temperature above 17°C ($rho = -0.98$, $p < 1e\text{-}4$, $N = 8$, Spearman rank correlation test). Evoked rhythms generated at higher temperatures were generally shorter in duration than those evoked at lower temperature within the same preparation (7/8 preps). These data show that the neural circuit controlling mastication can operate across a greater range of temperature than previously thought (Stadele et al. 2015) and better reflects the range of temperatures naturally encountered by *C. borealis*.

Disclosures: D.J. Powell: None. **S.A. Haddad:** None. **S. Gorur-Shandilya:** None. **M. Rue:** None. **E.E. Marder:** None.

Poster

230. Cells, Circuits, and Motor Patterns

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 230.05/O4

Topic: E.07. Rhythmic Motor Pattern Generation

Support: MH046742 and R35 NS097343 (EM)

T32 NS07292 and Swartz Foundation 2017 (LA)

Title: Temperature compensation in model pyloric networks

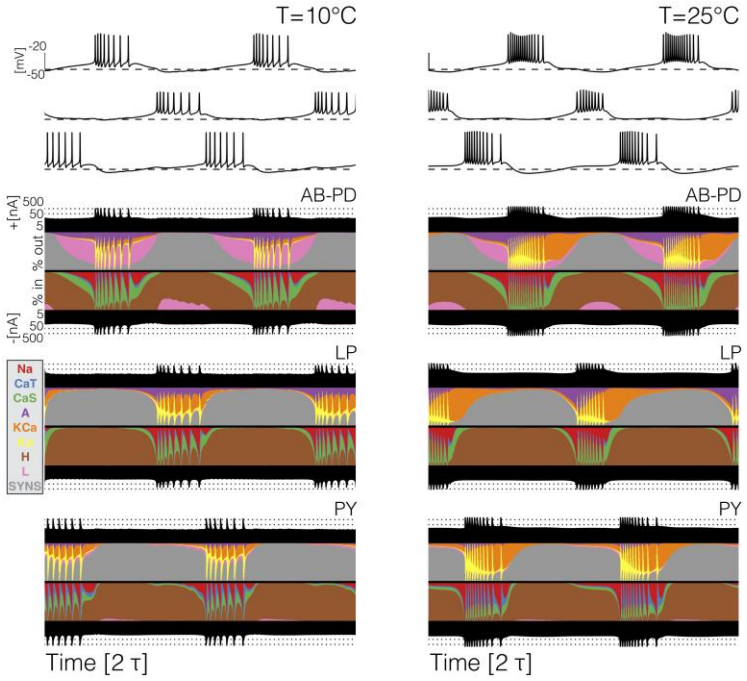
Authors: *L. M. ALONSO[1], E. MARDER[2];
[2]Volen Ctr. and Biol. Dept., [1]Brandeis Univ., Waltham, MA

Abstract: Temperature affects the conductances and kinetics of ionic channels that underlie neural activity. Intriguingly, experiments show conclusively that temperature has different effects on different ion channel types [Tang et al., 2010]. This raises the question of how neurons and neural circuits can operate robustly over wide temperature ranges. To address this, we employed computational models of the pyloric network of crabs and lobsters. We produced 36 different models that exhibit the pyloric rhythm over a range of temperatures and explored the dynamics of their currents and how they change with temperature. We found that temperature causes the currents to change their contributions to the activity in multiple different ways. In general, the currents that produce the rhythm contribute different ratios at different temperatures. As a consequence of this, the responses of the models to extreme perturbations -such as gradually decreasing a current type- are in most cases qualitatively different at different temperatures.

References: Tang, L. S., Goeritz, M. L., Caplan, J. S., Taylor, A. L., Fisek, M., and Marder, E. (2010). Precise temperature compensation of phase in a rhythmic motor pattern. PLoS biology, 8(8):e1000469.

Funding acknowledgments: Research supported by MH046742 and R35 NS097343 (EM), T32 NS07292 and Swartz Foundation 2017 (LA).

Figure caption: The contribution of each current to the total at each instant of time is represented in colors. The figure shows the how the currents change due to temperature.



Disclosures: **L.M. Alonso:** None. **E. Marder:** None.

Poster

230. Cells, Circuits, and Motor Patterns

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 230.06/O5

Topic: E.07. Rhythmic Motor Pattern Generation

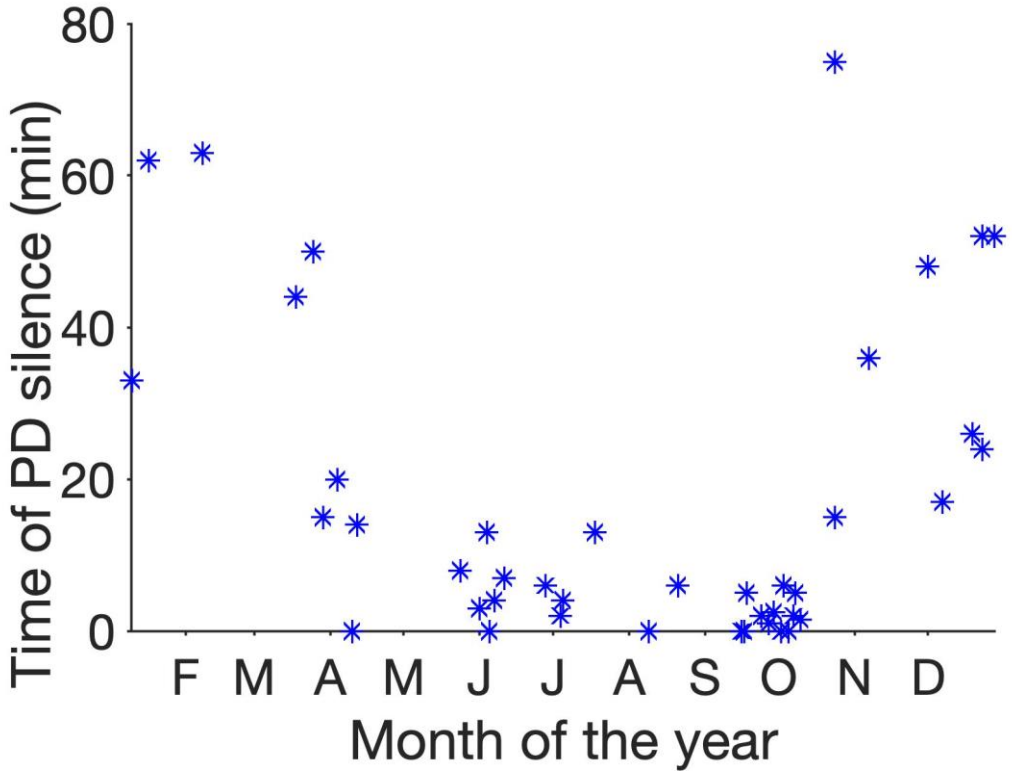
Support: NSF Grant R35 NS097343

Title: Seasonal variation in robustness to elevated extracellular potassium in pyloric neurons of the crab, Cancer borealis

Authors: *M. RUE[1], E. MOROZOVA[1], L. HE[1], D. J. POWELL[2], E. E. MARDER[3]; [2]Neurosci., [1]Brandeis Univ., Waltham, MA; [3]Volen Ctr. and Biol. Dept., Volen Ctr., Waltham, MA

Abstract: Neuronal circuits must be robust to various environmental challenges. This is especially true for central pattern generators (CPGs) that produce essential motor patterns such as walking, breathing and chewing (Marder and Calabrese, 1996). Recently, we characterized the effects of increased extracellular [K^+] on the well-described pyloric circuit of the crab, *Cancer borealis*, which drives the filtering of food through the foregut. A 2.5-fold increase in extracellular [K^+] depolarized Pyloric Dilator (PD) neurons and resulted in an unexpected loss of normal bursting activity and a period of silence in all pyloric neurons. This was followed by recovery of spiking and/or bursting during continued superfusion of 2.5x[K^+] saline via an increase in PD neuron excitability.

There is considerable individual variability in the timing and robustness of the response to elevated [K^+]. Across animals, the time until recovery of PD neuron action potentials varied from more than an hour to less than a minute. Much of this variability is correlated with the season in which experimental animals (N = 42) were collected; in warmer summer months PD neurons are more robust to elevated [K^+] and recover spiking activity sooner than PD neurons recorded from in the colder winter months. We next tested whether heat shock proteins, molecular chaperones associated with increased robustness to many cellular stressors, were upregulated in STG exposed to elevated [K^+]. STG exposed to 2.5x[K^+] for several hours (N = 8) had elevated levels of HSP70, 40 and 90 mRNA compared to STG exposed to only physiological saline (N = 5). Together, these data suggest that acute heat shock may affect the neuronal response to elevated [K^+] and contribute to seasonal variations in circuit robustness.



Disclosures: **M. Rue:** None. **E. Morozova:** None. **L. He:** None. **D.J. Powell:** None. **E.E. Marder:** None.

Poster

230. Cells, Circuits, and Motor Patterns

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 230.07/O6

Topic: E.07. Rhythmic Motor Pattern Generation

Support: Goethe Grant 100294
Faculty Development Grant CSUS

Title: Within and between animal variability in the firing properties of identified leech neurons

Authors: J. CHAO, A. LADRILLANO, A. SISON, V. PASMURTSEV, J. AMMARI, T. MCDOUGAL, C. PAYAN, ***T. M. WRIGHT, Jr**;
California State University, Sacramento, Sacramento, CA

Abstract: It is now well established that there is considerable animal to animal variability in the production of rhythmic motor patterns. Recent work in the medicinal leech, *Hirudo*

*medicinalis*has quantified the variability in the production of the motor pattern that drives the rhythmic constrictions of a bilateral pair of heart tubes. This variability begs the question: what parameters must be fixed in order to produce stereotyped behaviors despite the variability inherent within the nervous system? To address this question, we will explore variability in the firing properties of several identified leech neurons. A subset of the neurons that will be used in this study, the Heart Excitor (HE) motor neurons, underlie the rhythmic constriction of the Heart tubes. Other identified neurons, such as the mechanosensory T,P, and N cells, have been shown to have an influence over the activity of the heartbeat system, and thus variability in their firing properties, while not directly involved in the cycle-to-cycle variability of the heartbeat pattern may never the less contribute to that variability. Because the output of many neural circuits is the firing of action potentials, the variability in the generation of action potentials will contribute to the variability that we observe. The variability in firing properties will also inform us about the intrinsic properties of the neurons themselves, without an exhaustive measurement of all of the currents that flow across the membrane. To do this, we recorded from several neuron types in adult leeches. We used several current injection protocols designed to assess the firing properties of the neurons. For example, we injected a sequence of current steps to generate Frequency-Current (FI) curves. We then assessed the shape of these curves to determine whether individual neuron types exhibit Type 1 (N-cell) or 2 (P-cells) spiking behavior. Within those Type 1 and 2 curves, we then characterized the animal-to-animal variability in parameters of those FI curves, including average firing rates at a given current injection amplitude as well as the slope of the relationship between firing frequency and current for a given neuron. Other current injection protocols were also be used to assess firing properties of neurons, including whitenoise and sinusoidal inputs. We show that different cell types exhibit different filtering properties, including low-pass (HE cells) and some aspects of hi-pass filtering (P, T cells).

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Poster

230. Cells, Circuits, and Motor Patterns

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 230.08/O7

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant MH46742

Title: Molecular and physiological correlates of neuronal cell identity in a small central pattern generating network

Authors: *A. J. NORTHCUTT[1], D. R. KICK[1], A. G. OTOPALIK[2], B. M. GOETZ[3], J. SANTIN[4], H. A. HOFMANN[3,5], E. MARDER[6], D. J. SCHULZ[1,5];

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Abstract: Currently there is a major push to identify, categorize, and subdivide populations of neurons, particularly in the mammalian brain. An attractive method commonly employed to differentiate neurons into subgroups is molecular profiling of single cell transcriptomes. However, it is unclear how well neurons can be accurately classified without *a priori* anatomical or physiological information. The stomatogastric (STG) and cardiac (CG) ganglia of the crustacean *Cancer borealis* contain well-characterized, unambiguously identifiable classes of motor neurons that we can use to tackle this problem. Here we exploit this system to determine how well cell types from known endpoints of neuron identity can be recovered by molecularly profiling individual neurons. We used open-ended (single cell RNA-sequencing) and candidate gene (RT-qPCR) approaches to analyze the molecular profiles of over 200 identified neurons across 11 well-studied cell types from small circuits in *Cancer borealis*. We then explored how well various clustering methods can correctly classify the different cell types contained in each data set. Hierarchical clustering and supervised machine learning algorithms performed with much lower accuracy when cell identity information was restricted, and subsequently improved when differential expression analyses could be employed. Importantly, in the absence of *a priori* anatomical or physiological information these analyses failed to accurately recover cell identity for all 11 cell types. We further show how gene co-expression network analyses can be used to discriminate neuron types beyond simply comparing their mRNA abundances directly. Unexpected co-expression patterns were revealed, such as strong correlations between *vGluT* (a marker for glutamatergic neurotransmitter phenotype) and *CCAPr* (crustacean cardioactive peptide receptor). Finally, we compared these results to the known electrophysiological properties of these neurons to identify correlates between expression patterns and components of neuronal activity, such as the *HCN/IH* hyperpolarization-activated channel mRNA being 118-fold higher in the intrinsically oscillating AB pacemaking neuron than other cell types. In sum, our results demonstrate the limitations of single cell molecular profiling in the absence of anatomical or physiological information and the importance of an integrative approach to cellular neuroscience.

Disclosures: A.J. Northcutt: None. **D.R. Kick:** None. **D.J. Schulz:** None. **H.A. Hofmann:** None. **A.G. Otopalik:** None. **B.M. Goetz:** None. **J. Santin:** None. **E. Marder:** None.

Poster

230. Cells, Circuits, and Motor Patterns

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 230.09/O8

Topic: E.07. Rhythmic Motor Pattern Generation

Title: Detailed biologically realistic model of a crustacean cardiac ganglion network

Authors: *D. DOPP, T. BANKS, P. SAMARTH, D. KICK, D. SCHULZ, S. NAIR;
Univ. of Missouri, Columbia, MO

Abstract: The crustacean cardiac ganglion (CG) network coordinates the rhythmic contractions of the heart muscle to control the circulation of blood. The network consists of 9 cells, 5 large motor cells (LCs) and 4 small endogenous pacemaker cells (SCs). Variability in maximal conductances of intrinsic currents has been reported for the five LCs, even within the same animal. Despite this variability, the intact network maintains a synchronous output, but how it does so is unclear. Computational models of LCs used to explore this issue have been limited to single compartmental configurations. Here we report a three-compartmental model comprising a soma, neurite, and a spike-initiation zone (SIZ) with the SC input at the SIZ compartments. Biological data for the model comes largely from the Schulz Lab, including first-hand recordings of intrinsic currents from LCs of C. Borealis.

We initially focus on the distribution of conductances in the soma and neurite compartments of the 3-compartmental LC model. Experimental data for this development uses electrophysiological data from control and TEA-perfused conditions for both ligated soma and for intact cells in a network. A systematic exploration within the biological ranges of the conductance space for the known currents in LCs revealed that the neurite compartment requires active conductances, specifically, persistent sodium conductances in neurites, as well as Ca conductances to reproduce the waveform variety seen in biological data.

A second focus of the study is on exploring possible conductance covariations for this revised CG LC model that would preserve both cellular and network output. Cellular output will include the single cell properties listed above, spikes per burst, and spike frequency. For the cellular output case, we use a rejection sampling technique to form a pool of acceptable parameter sets in two steps. First, we select maximal conductance values for nine currents from a biologically constrained 9-dimensional parameter space, and simulate the model. Second, if the model output replicates features within known bounds for the cellular outputs cited, then the parameter set is retained. Using the parameter sets generated by the rejection sampling scheme, we investigate conductance covariations that exist naturally in the dataset. We will then use the single cell models that pass the rejection protocol to create a network of cells. The creation of this network will introduce the parameter for gap-junction coupling between the SIZs. The analysis of the network output will be performed using another rejection protocol that will add the additional feature of inter-burst interval.

Disclosures: D. Dopp: None. **T. Banks:** None. **P. Samarth:** None. **D. Kick:** None. **D. Schulz:** None. **S. Nair:** None.

Poster

230. Cells, Circuits, and Motor Patterns

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 230.10/O9

Topic: E.07. Rhythmic Motor Pattern Generation

Support: DFG EG 401/1-1
NIH R01 NS075044

Title: Impact of an inhibitory network onto individual premotor neurons with single synapse resolution *in vivo*

Authors: *R. EGGER[1], M. A. LONG[2];
[2]Neurosci. institute, [1]NYU Sch. of Med., New York, NY

Abstract: Sequential neuronal activity patterns form the network basis for a diverse range of neural processes across various brain regions. For instance, such sequences have been observed in the hippocampus during spatial exploration and mental replay, in the striatum during movement planning, and in the parietal cortex during decision-making. However, the cellular and network mechanisms underlying sequence generation in forebrain circuits are at present not well understood.
The zebra finch has emerged as an excellent model system for studying sequential network dynamics underlying a complex learned behavior. In a key cortical structure, HVC premotor neurons produce a sequence of bursting activity, with each individual neuron active at only one point throughout the sequence. Local circuit interneurons, the sole source of inhibition in HVC, have been proposed to be important for shaping the dynamics of the ongoing premotor sequence. To examine the role of HVC interneurons in generating network sequences, we introduce MERgE (MultiElectrode Recording/conductance[g] Electrophysiology), a hybrid electrophysiological approach. Specifically, we combine silicon probe measurements of spiking activity of a population of dozens of simultaneously recorded interneurons with whole-cell voltage-clamp recordings of inhibitory synaptic inputs onto a single premotor neuron. We perform a spike-triggered average of the IPSC trace for each interneuron, revealing a number of putative monosynaptic connections. We then ask how each interneuron is contributing to the inhibitory profile converging onto premotor neurons, enabling a direct observation of these synaptic inputs that are thought to maintain and support large-scale sequential activity.

Disclosures: R. Egger: None. **M.A. Long:** None.

Poster

230. Cells, Circuits, and Motor Patterns

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 230.11/O10

Topic: E.07. Rhythmic Motor Pattern Generation

Support: R01 NS075044
T32 NS086750-05S1

Title: Establishing behaviorally relevant subclasses of GABAergic interneurons within a songbird premotor circuit

Authors: *E. HOZHABRI[1], M. A. LONG[2];
[1]Neurosci., NYU Sch. of Med., New York City, NY; [2]Neurosci. institute, NYU Sch. of Med., New York, NY

Abstract: GABAergic interneurons enrich the computational power of neural circuits, with different inhibitory subtypes often playing distinct roles within a network. In the mammalian brain, these subpopulations can be defined based on their morphological, physiological, molecular, and connectivity profiles. Although these classes have been well characterized in sensory cortices, the roles of distinct inhibitory cell types in the production of complex motor behaviors are still largely unknown.
To address this issue, we investigated the role of GABAergic interneurons within the zebra finch song production pathway, which has the distinct advantage of a complex, but highly tractable, behavior mediated by set of well-characterized brain regions. In a key premotor locus, called HVC, our lab had previously shown that inhibition - mediated by local circuit interneurons - plays several distinct roles in song learning and production. Furthermore, we and others have characterized heterogeneous anatomical and electrophysiological properties of HVC interneurons. Here we begin to test the hypothesis that HVC interneurons can be further classified into functional subclasses that play distinct roles in that network.
To characterize different functional subcategories of HVC interneurons, we used the AAV-mDlx-GFP virus, capable of targeting the vast majority of GABAergic neurons within HVC. We then characterized the activity of labeled neurons in two ways. First, we measured spiking using *in vivo* cell-attached recordings under 2-photon guidance to create an electrophysiological profile for each neuron, combining both passive and sensory-driven activity. Second, we expressed the genetically encoded calcium indicator GCaMP6f in these neurons to examine activity in head-fixed singing birds with single-neuron resolution. Upon completion of these experiments, neurons were recovered and assayed for common molecular markers (e.g., somatostatin and parvalbumin) and their morphological properties were further characterized in an effort to establish categorical distinctions between interneurons within the context of behavior.

Disclosures: **E. Hozhabri:** None. **M.A. Long:** None.

Poster

230. Cells, Circuits, and Motor Patterns

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 230.12/O11

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant R01 NS075044

Title: Imaging axons of motor thalamus in HVC of the singing zebra finch

Authors: *F. W. MOLL, D. KRANZ, M. A. LONG;
Neurosci. Inst., NYU Sch. of Med., New York, NY

Abstract: Thalamic input to motor cortex has been shown to be essential for simple skilled movements. However, its role in learned complex behaviors is poorly understood. The courtship song of the zebra finch is a well studied skilled motor-behavior that consists of one learned, stereotyped sequence of syllables. The song is represented on a moment-to-moment basis by a sequence of sparse activity in the forebrain song nucleus HVC. A key upstream region of HVC and its sole thalamic input is the small nucleus Uvaeformis (Uva). Previous lesion studies have indicated the importance of Uva for singing behavior; bilateral lesions of Uva mute a songbird permanently without impacting its motivation to sing to a female. Furthermore, multiunit recordings have shown that Uva neurons are strongly modulated during singing, but the role of single Uva neurons projecting to the forebrain song nucleus HVC remains elusive. Two main models have been proposed to explain the role of Uva-HVC projection neurons for song generation: (1) Uva is part of a distributed loop in which rapid cycling of activity through several brain regions is necessary to advance the song on a moment-to-moment basis. (2) The representation of song timing is localized to HVC, while Uva is performing a necessary supportive role, perhaps initiating syllables. To begin to address these models, we used in vivo 2-photon imaging combined with the fluorescent activity indicator GCaMP7f to look at the song-related activity of Uva axons in HVC. Axon terminals of single Uva(HVC) projection neurons exhibited patterned calcium transients throughout singing, and initial results suggest that this activity correlates with syllable onsets. We then asked whether Uva preferentially targets a functional subgroup of HVC neurons active at the beginning of syllables, we virally labeled HVC neurons with GCaMP7f and registered neurons that got activated by focal Uva stimulation. To establish the role of these neurons within the song sequence, we examined the activity of the Uva-triggered neurons during singing. Taken together, our results can help to characterize the role of thalamic inputs in skilled motor performance.

Disclosures: **F.W. Moll:** None. **D. Kranz:** None. **M.A. Long:** None.

Poster

230. Cells, Circuits, and Motor Patterns

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 230.13/O12

Topic: E.07. Rhythmic Motor Pattern Generation

Support: R01 DC015260

Title: The role of inferior frontal gyrus in speech planning for rapid conversational turn-taking

Authors: *G. A. CASTELLUCCI[1,2], J. D. GREENLEE[3], M. A. LONG[1];
[1]Neurosci. Inst., NYU Langone Med. Ctr., New York, NY; [2]Haskins Labs., New Haven, CT;
[3]Neurosurg., Univ. of Iowa, Iowa City, IA

Abstract: During conversation, speaker overlap is avoided while silent gaps between turns are minimized. Notably, inter-speaker gaps are typically 200 ms or less, which is shorter than the time required to initiate the production of a word in response to a cue. To achieve this degree of temporal precision, significant speech planning must occur while a speaker listens to their partner's turn. Though the psycholinguistic mechanisms of this behavior have been studied in detail, the neural dynamics underlying speech planning during turn-taking are largely undefined. Using electrocorticography (ECoG) in neurosurgical patients, we isolate this planning process using a series of questions (adapted from Bögels *et al.*, 2015) in which the critical information (CI) needed to produce an answer is provided only at a single timepoint. We therefore hypothesize that the cortical sites involved in speech planning will modulate their activity immediately following CI presentation. We observe that left inferior frontal gyrus (IFG; "Broca's area") displays increased high gamma power in response to CI. In a separate task, we find that CI-related IFG activity is not observed during planning for general motor behavior (hand and orofacial movement) or simple speech repetition, but rather for tasks requiring complex linguistic processing (pluralization). Furthermore, we observe that a subset of CI-active IFG planning sites are also active during natural conversation while patients listen to the opposing speaker's turns. To causally implicate these IFG sites in turn-taking behavior, we have begun stimulation experiments where direct cortical stimulation is applied to CI-active planning sites while patients answer CI-questions. In these initial experiments, we find that stimulating IFG results in significantly longer reaction times as well as lexical errors and hesitations, but not speech arrest or gross articulatory disruptions. In conclusion, we demonstrate that a subregion of IFG is critical to the planning processes underlying rapid turn-taking in conversation, and that this region is essential for high-level linguistic planning.

Disclosures: G.A. Castellucci: None. **J.D. Greenlee:** None. **M.A. Long:** None.

Poster

230. Cells, Circuits, and Motor Patterns

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 230.14/O13

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH T32MH019524-26
Simons Collaboration on the Global Brain

Title: Understanding the neural substrates of sequence generation through sleep replay

Authors: *M. ELMALEH[1], M. A. LONG[2];
[1]NYU Langone Med. Ctr., New York, NY; [2]Neurosci. Inst., NYU Sch. of Med., New York, NY

Abstract: Sequential neural activity has been observed in many brain regions and has been proposed to underlie a range of functions. In the songbird, a key premotor cortical area (HVC) generates a population-level sequence that mediates the performance of the adult courtship song. Zebra finch singing behavior is composed of a series of 3-5 repeated complex vocal elements (duration: ~200 ms) known as syllables. Behavioral and lesion studies have suggested that the syllable is the minimum basic unit of the song. However, it remains unknown whether HVC is composed of discrete syllable-generating circuits or a single continuous network in which the entire behavior is embedded. One challenge to understanding the circuit mechanisms that give rise to the HVC sequence is that multiple brain regions are active in a highly correlated fashion during singing, making it difficult to understand the individual contributions of these areas. A qualitatively different situation exists during sleep, where specific brain regions appear to 'replay' fragments of the song in the absence of vocal production. Here we use high-density silicon probes to simultaneously record a large population of neurons in the robust nucleus of the arcopallium, a region downstream of the premotor sequence generator, enabling us to precisely decode the song content of these replay events. We find that while sequences across hemispheres are highly synchronized during song, sleep replay exists on the left and right side independently. Within a single hemisphere, replay snippets often cross syllable boundaries, suggesting that the underlying circuitry within HVC is unlikely to consist of separate subpopulations dedicated to individual syllables.

Disclosures: M. Elmaleh: None. **M.A. Long:** None.

Poster

230. Cells, Circuits, and Motor Patterns

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 230.15/O14

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant NS094176
Regenerative Medicine Minnesota

Title: Comparison of canonical and novel stimulation paradigms that produce spinally-mediated locomotor activity

Authors: *J. E. MONTGOMERY, S. WAHLSTROM-HELGREN, K. T. VANPELT, M. A. MASINO;
Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Pattern-generating neural circuitry is responsible for controlling the rhythmic outputs of a variety of essential functions, such as respiration and locomotion. Given a source of excitation, spinal locomotor networks have the intrinsic capacity to generate coordinated locomotor output, permitting investigation of these pattern-generating circuits in isolation from the brain. Systemic bath application of the excitatory chemical N-methyl-D-aspartate (NMDA) has been an effective method of activating spinal networks to produce locomotor activity. However, our understanding of the mechanisms through which NMDA produces this activity remains unclear, which may be a consequence of our incomplete understanding of locomotor networks as a whole. The development of novel spatially- and temporally-controllable network stimulation paradigms will facilitate our understanding of the circuitry underlying the production of rhythmic neural activity.
In the present study, *Channelrhodopsin-2* was expressed in spinal glutamatergic neurons of larval-stage zebrafish. Since larval zebrafish are transparent, these neurons were activated by applying a constant (10 s) epifluorescent, blue-light stimulus to spinally-transected preparations. Despite simultaneous and continuous stimulation of multiple subtypes of glutamatergic neurons, the activity of these neurons still organized to produce coordinated fictive swimming throughout the duration of each stimulus. Fictive swimming properties were similar to spontaneous fictive swimming in intact larvae and NMDA-induced swimming in spinally-transected larvae, although optogenetically-induced fictive swimming frequency (22.0 Hz, SD 1.5) was higher than NMDA-induced swimming frequency (14.2 Hz, SD 2.5) and lower than intact, spontaneous swimming frequency (26.7 Hz, SD 4.0). Next, we compared the changes in fictive swimming properties over extended time-courses (~60 min). The amount of optogenetically-induced fictive swimming activity (number of peripheral motor nerve bursts) produced during individual blue light stimuli decreased over successive stimulations, while the amount of NMDA-induced fictive swimming

activity did not change. However, the organization of activity into discrete episodes of swimming decreased over time during NMDA-induced swimming, while optogenetically-induced swimming did not change. Thus, each method of network activation possesses characteristics to be explored and accounted for when employing them.

Disclosures: J.E. Montgomery: None. **S. Wahlstrom-Helgren:** None. **K.T. Vanpelt:** None. **M.A. Masino:** None.

Poster

230. Cells, Circuits, and Motor Patterns

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 230.16/O15

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH RO1 NS094176 (MAM)
Regenerative Medicine Minnesota (JEM)

Title: Optogenetic activation of spinal glutamatergic neurons elicits swimming in zebrafish larvae

Authors: *S. WAHLSTROM-HELGREN, J. E. MONTGOMERY, K. T. VANPELT, S. L. BILTZ, M. A. MASINO;
Univ. of Minnesota, Minneapolis, MN

Abstract: The spinal cord (SC) contains neural networks that are capable of producing organized locomotor activity autonomously from the brain. To study the production of locomotor activity from spinal circuits in isolation from the brain, it is necessary to transect the SC. Locomotor activity can be induced in spinally-transected (spinalized) animals by adding a source of tonic excitation to activate spinal networks. This is commonly accomplished to by activating N-methyl-D-aspartate (NMDA) glutamate receptors through bath application of NMDA. More recently, optogenetic approaches have enabled both activation and inactivation of neuronal cell populations to control the activity of locomotor networks. Larval zebrafish are exceptionally amenable to optogenetic techniques due to their transparency, which permits noninvasive light delivery. Here, we induced locomotor activity in spinalized transgenic zebrafish larvae that expressed *channelrhodopsin-2* in all subtypes of spinal glutamatergic neurons by applying 10 s of constant blue light to the preparations. The resultant locomotor activity possessed all of the characteristics of coordinated fictive- and free-swimming: bilateral alternation, rostrocaudal progression, and organization into discrete swimming episodes. Spatially-restricted light application revealed that illumination of the rostral SC produced more robust activity than illumination of the caudal SC. Moreover, illumination of only three body segments was sufficient to produce fictive swimming. Intriguingly, pharmacological block of NMDA receptors

did not affect the amount of locomotor activity produced during blue light stimulation. Using this novel spatially and temporally-controlled approach, we demonstrate that activation of glutamatergic spinal neurons elicits coordinated and organized swimming in spinalized zebrafish larvae.

Disclosures: S. Wahlstrom-Helgren: None. **J.E. Montgomery:** None. **K.T. Vanpelt:** None. **M.A. Masino:** None. **S.L. Biltz:** None.

Poster

230. Cells, Circuits, and Motor Patterns

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 230.17/O16

Topic: A.08. Development of Motor/ Sensory/ and Limbic Systems

Support: NIH R01 NS 047357
NIH R21 NS 106209

Title: Development of a dual-conditional transgenic mouse model for genetic targeting of Renshaw cells in adult animals

Authors: *A. R. LANE, F. J. ALVAREZ;
Physiol., Emory Univ., Atlanta, GA

Abstract: Renshaw cells (RCs) are interneurons that inhibit motoneurons (MNs) and receive direct input from recurrent collaterals of motor axons as they exit the spinal cord. This recurrent circuit is the oldest inhibitory circuit known in the mammalian CNS, but it is unclear what functions it mediates during motor behavior. Known connections of RCs—MNs and Ia inhibitory interneurons—suggest several roles, including limitation and decorrelation of MN firing, focusing Ia activation to specific motor pools, regulating motor input-output gains, and adjusting joint stabilization and co-contraction through actions on IaINs; however, these roles are yet to be validated due to the difficulty of isolating RC activity from other network elements. Recent advancements on the genetic fingerprint of RCs opened possibilities for genetic targeting. We focused on calcium-buffering proteins, using mice carrying a trimethoprim (TMP) inducible *dgCre* variant controlled by the calbindin (*calb1*) promoter to genetically label RCs. Up to 99% of RCs were labeled following TMP administration, with significant off-target labeling predominating in the dorsal horn. We then vetted *calb2* (calretinin) and *pvalb* (parvalbumin) as candidates to further restrict targeting to RCs and found that both are expressed in approximately 60% of RCs at P21. Crossing *calb1-dgCre* and *pvalb-flp* mice produced dramatic reductions in off-target labeling, with maximum RC labeling of 93%. Non-RC labeled spinal interneurons were scattered primarily throughout the dorsal horn, with very few in the ventral horn. Additional genetic labeling was detected in cerebellar Purkinje cells, superior olive and reticular

thalamic interneurons, and a few other regions with scattered cells. To further restrict targeting to the spinal cord, an AAV9 dual-conditional reporter virus was injected into the caudal lumbar spinal cord at P5. Labeled interneurons are found throughout the lumbar region, predominantly on the side ipsilateral to the injection, with minimal labeling in adjacent sacral and thoracic regions. These results constitute the first genetic targeting of RCs to bypass development and are currently in use to direct expression of activity modifiers (Gi DREADD, tetanus neurotoxin subunit a, and others) in RCs.

Disclosures: **A.R. Lane:** None. **F.J. Alvarez:** None.

Poster

230. Cells, Circuits, and Motor Patterns

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 230.18/O17

Topic: A.08. Development of Motor/ Sensory/ and Limbic Systems

Support: NIH F31 NS110235
NIH T32 Training Grant #5T32NS086750
NIH T32 Training Grant 5T32MH096331

Title: Evi1 and Prdm16 define motoneuron subtypes necessary for fast/intermediate speed swimming

Authors: ***K. P. D'ELIA**[1], D. SCHOPPIK[2], J. DASEN[1];
[1]Neurosci. Inst., New York Univ. Sch. of Med., New York, NY; [2]Otolaryngology and Neurosci. & Physiol., New York Univ. Langone Med. Ctr., New York, NY

Abstract: Locomotion requires the precise control of the strength and speed of muscle contraction. Precision is achieved by recruiting subtypes of motoneurons (MNs) that contract muscle fibers with different functional properties. These subtypes of MNs are essential to movement and differentially susceptible in disease, but we know almost nothing about their development and how they acquire specific functional properties such as firing patterns and role in behavior. Combining single-cell genomics, cellular analysis, and behavior, I have investigated the genetic programs underlying functional MN subtypes in zebrafish. Since little is known about the determination of MN subtypes in zebrafish, I used single-cell RNA sequencing (scRNA seq) to evaluate if these populations are molecularly distinct. I was able to distinguish MN clusters based on their transcriptomes, procuring a list of potential subtype markers. These markers provide genetic handles into diverse neuronal subtypes allowing the creation of tools necessary to determine their molecular development and role in locomotion. We identified two possible functional subtype-labeling transcription factors, Evi1 and Prdm16. Interestingly, I found that Evi1 and Prdm16 expression is highly conserved in MNs across multiple vertebrate species,

including zebrafish, mouse, chicken, and skate. This suggests Evi1 and Prdm16 may play conserved roles in axial MN subtype specification across evolution. By creating an antibody and pairing it with sparse MN labeling, I determined Evi1 and Prdm16 label four distinct anatomical MN subtypes in zebrafish likely involved in fast/intermediate speed swimming. To investigate whether Evi1 and Prdm16 might have a role in the specification of these functional subtypes, I used Crispr/Cas9 to create loss-of-function mutant lines. I have found that removal of Evi1 leads to slower swimming in mutants without gross loss of either MNs or muscle innervation. This behavioral phenotype may indicate these transcription factors are necessary for proper development of a subclass of motoneurons responsible for faster speeds of swimming. Together, these results give insight into the molecular determination of functional MN subtypes.

Disclosures: **K.P. D'Elia:** None. **D. Schoppik:** None. **J. Dasen:** None.

Poster

230. Cells, Circuits, and Motor Patterns

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant NS17323
France Grant CalpaSCI
France Grant ANR-16-CE16-0004
France IRME

Title: A size principle dictates bistability in neonatal mouse alpha motoneurons

Authors: *R. M. HARRIS-WARRICK[1], R. BOS[2], F. BROCARD[3];
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Abstract: When studied under normal physiological conditions (ACSF with 1.2 mM $CaCl_2$, temperatures above 30°C) many neonatal (P1-P14) motoneurons exhibit bistable firing behavior. This is characterized by a common set of properties including: 1) prolonged firing, or a prolonged afterdepolarization, following a brief intense stimulus; 2) negative hysteresis during ramp current injections; 3) slow depolarizations during subthreshold current steps, leading to delayed onset and acceleration of spike frequency. However, not all motoneurons show these properties: bistable behavior varies markedly depending on the size of the motoneuron. We studied this with Hb9-GFP mice, which label alpha but not gamma motoneurons, in the ventrolateral lower lumbar spinal cord; similar results were seen with ChAT-GFP mice. 76% of small motoneurons (less than 20µm diameter, input resistance &gt300 MOhms) show no bistable properties; many of the younger neurons (<P5) fire phasically during current steps, and only 7%

showed bistable activity. 57% of large motoneurons (&gt30µm diameter, input resistance &lt100MOhms) were fully bistable, with only 11% lacking any bistable properties. Intermediate motoneurons (20-30µm diameter, 100-300 MOhms Input resistance) show a variable subset of bistable properties: 68% showed one or more bistable properties but only 28% were fully bistable. With developmental maturation, an increasing percentage of all motoneurons are bistable; however, bistability is still limited to large and intermediate motoneurons, with small motoneurons failing to show any bistable properties. Serotonin (5-10µM) can transform intermediate neurons into fully bistable neurons. However, serotonin is unable to enhance any bistable properties in small motoneurons. The large motoneurons probably belong to the F-type class that drives fast-contracting muscles, while smaller motoneurons belong to the S-type class driving slow-contracting muscles. Thus, bistability is predominantly limited to large, likely F-type motoneurons during early maturation. Since S-type motoneurons play a major role in maintenance of posture, our results suggest that bistability does not play a major role in postural control at this age. Supported by NIH NS17323, CalpaSCI, ANR-16-CE16-0004 and IRME.

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Poster

230. Cells, Circuits, and Motor Patterns

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: Wellcome Trust Grant 109002/Z/15/A

Title: Examining the effect of V3 interneurons and astrocytes on embryonic stem cell-derived motoneuron maturation *in vitro*

Authors: *A. H. HANDS, J. B. BRYSON, R. BROWNSTONE, L. GREENSMITH;
Univ. Col. London, London, United Kingdom

Abstract: Current methods for restoring function to paralysed muscles rely mainly on stimulation of host nerves. However, this is only effective in cases where motoneurons and neuromuscular junctions (NMJs) are intact, which may not be the case in disorders such as amyotrophic lateral sclerosis. When transplanted into a peripheral nerve, embryoid bodies (EBs) containing embryonic stem cell (ESC)-derived motoneurons can form functional NMJs, enabling control of muscle contraction by electrical or optical stimulation of the graft. However, if purified motoneuron aggregates are used, functional NMJs do not form. Therefore, other cells within EBs seem to contribute to the ability of ESC-derived motoneurons to mature and functionally innervate host muscle. We hypothesised that spontaneous activity arising from intra-graft microcircuits is necessary for motoneuron maturation. **Methods:** To investigate this

hypothesis, I generated *in vitro* co-cultures with pure populations of ESC-derived motoneurons, astrocytes and V3 interneurons. Whole-cell patch clamping and calcium imaging were used to investigate the electrophysiological properties and spontaneous activity of motoneurons cultured alone or in combination with other cell types. Immunohistochemistry was used to assess effects of co-culture on motoneuron morphology and synapse formation. **Results:** Co-culture of motoneurons with astrocytes accelerated morphological and electrophysiological motoneuronal development, the formation of glutamatergic and cholinergic synapses, and the development of glutamate-dependent, motoneuronal spontaneous activity which was modulated by cholinergic signalling. When V3 interneurons were added to the motoneuron/astrocyte co-cultures, development and maturation of motoneuronal spontaneous activity was accelerated and the number of glutamatergic and cholinergic synapses on to the cell bodies of motoneurons were increased and decreased, respectively. **Conclusions:** These results provide insight into the role of other cell types in motoneuron maturation and suggest that astrocytes and V3 interneurons could be added to motoneurons to produce a more mature, spontaneously active graft.

Disclosures: A.H. Hands: None. **J.B. Bryson:** None. **R. Brownstone:** None. **L. Greensmith:** None.

Poster

230. Cells, Circuits, and Motor Patterns

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 230.21/O20

Topic: E.07. Rhythmic Motor Pattern Generation

Support: CIHR Grant 14392

Title: Synchronization and coordination of neuronal firing by astrocytes in the chewing central pattern generator

Authors: *M. COUILLARD-LAROCQUE, S. CONDAMINE, A. KOLTA;
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Abstract: Chewing is a rhythmic motor activity generated by a neural network called Central Pattern Generator (CPG). Our previous work has shown that the trigeminal main sensory nucleus (NVsnpr), thought to be part of this CPG, contains neurons that fire rhythmically and that astrocytes and their protein S100ß were responsible for neuronal rhythmogenesis. If these neurons are responsible for the rhythmic motor command transmitted to motoneurons controlling jaw muscles, then they need to be synchronized.
Astrocytes couple to form well defined networks. We hypothesized that these networks may serve to synchronize ensemble of NVsnpr neurons innervating subgroups of motoneurons, since jaw muscles like the masseter have a particularly complex contraction pattern with different

compartments contracting at different times. We used whole cell recording, calcium imaging and optogenetic stimulation in brainstem slices of 10 days to 21 days old mice to test this hypothesis. We first found that stimulation of the axons of primary afferents projecting to NVsnpr, at a frequency that corresponds to their natural firing frequency during chewing (40 Hz) elicited synchronized firing in neurons recorded close to each other (n=2), but not those separated by more than 150 μm (n=2).
Interestingly, the same stimulation also activated NVsnpr astrocytes, as shown by an increase in intracellular Ca^{2+} in astrocytes expressing a calcium indicator (GCaMP6f) under the astrocytic promoter GFAP (n=1). To investigate if this activation could be the cause of the synchronization of groups of neighbouring neurons in NVsnpr, we used mice expressing a light sensitive channel, channelrhodopsin-2, under the astrocytic promoter S100β. Using this mouse, we found that optogenetic activation of NVsnpr astrocytes can induce firing in silent neurons (n=5), increase the discharge frequency of those firing spontaneously (n=3) or even elicit bursting (n=1). In paired recordings (n= 2), optogenetic activation caused synchronised firing in neurons close to each other (n=1), but not in neurons distant from each other (n=1). These results suggest that astrocytes may play a key role in patterning complex chewing movements, not only by generating rhythmic firing of NVsnpr neurons, but also by synchronizing their firing.

Disclosures: M. Couillard-Larocque: None. **S. Condamine:** None. **A. Kolta:** None.

Poster

231. Respiration: Modulation and Regulation

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 231.01/O21

Topic: E.08. Respiratory Regulation

Title: Comparison of physical properties of coughing and huffing in healthy humans

Authors: *T. TSUJIMURA, A. YAWATA, K. NAGOYA, M. INOUE;
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Abstract: Introduction: Voluntary coughing and huffing are known to be one of the manoeuvers in dysphagic patients to prevent aspiration, that are useful for sputum volume clearance in the airway. However, little is known about the relation of the physical properties between coughing and huffing. The aim of the present study was to compare the physical properties of them in healthy humans. **Methods:** Ten healthy volunteers were asked to coughing and huffing (i.e. a forced expiration with an open glottis) voluntarily in three separate trials. We recorded electromyograms (EMGs) from external intercostal (Inspiratory), sternocleidomastoid (Inspiratory), external oblique (Expiratory), submental (Suprahyoid) and thyrohyoid (Infrahyoid) muscles and airflow with a face mask with two-way non-rebreathing valves connected to the inspiratory and expiratory ports. We evaluated the reproducibility of these parameters using

intraclass correlation coefficient (ICC). The comparison and correlation among them were also investigated. **Results:** Basically, peak expiratory flow, expiratory volume and area under the curve of external intercostal, sternocleidomastoid and thyrohyoid EMGs in coughing and huffing, and submental EMG in coughing represented high ICC (>0.8). Peak inspiratory flow was positively correlated with peak expiratory flow in huffing but not coughing. Although peak expiratory flow was not correlated between coughing and huffing, expiratory volume of huffing was positively correlated with that of coughing. Furthermore, peak expiratory flow was not different between them and expiratory volume in huffing was significantly larger than that in coughing. Thyrohyoid EMG in coughing was significantly higher than that in huffing. Other EMGs were not significantly different between them. **Conclusion:** Both coughing and huffing showed high intraindividual reproducibility in expiratory airflow and inspiratory and thyrohyoid EMGs. There are some differences between coughing and huffing in expiratory airflow and thyrohyoid EMG. We speculate that these differences may be attributed to compression phase of cough.

Disclosures: T. Tsujimura: None. **A. Yawata:** None. **K. Nagoya:** None. **M. Inoue:** None.

Poster

231. Respiration: Modulation and Regulation

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 231.02/O22

Topic: E.08. Respiratory Regulation

Support: CAPES
CNPq
FAPESP (2015/23467-7)

Title: Effects of water deprivation on respiratory motor outputs during hypercapnia

Authors: E. F. SILVA, L. B. NAKAI, J. V. MENANI, D. S. A. COLOMBARI, D. B. ZOCCAL, *E. COLOMBARI;
Physiol. and Pathology, Sch. of Dent. of Araraquara - UNESP, Araraquara, Brazil

Abstract: It is known that osmolarity challenges produces reflex adjustments in cardiorespiratory function. Herein, we investigated whether 48 h of water deprivation (WD) modifies respiratory motor output under resting and hypercapnia conditions. Using decorticated, arterially-perfused *in situ* preparations, we recorded the activity of phrenic (PN), hypoglossal (HN), central vagus (cVN) and lumbar abdominal (AbN) nerves of male juvenile Holtzman rats (60-90 g) submitted to WD for 48 hs (WD, n=9). Control rats (n=11) had full access to water. Recordings were performed during normocapnia (5% CO_2) and hypercapnia (8% CO_2), while the *in situ* preparations were perfused with isosmotic (control rats: 306 ± 0.5 mOsmol/kg/water) or

hyperosmotic (WD rats: 321 ± 1.7 mOsmol/kg/water) solutions. The *in situ* preparations from WD rats exhibited increased PN bursts frequency (25 ± 1 bpm, *vs.* control: 15 ± 1 bpm; $p<0.05$), reduced PN (33 ± 2 µV, *vs.* control: 46 ± 4 µV; $p<0.05$) and HN burst amplitudes (22 ± 2 µV, *vs.* control: 39 ± 4 µV; $p<0.05$), reduced cVN activity during inspiration (8 ± 0.6 µV.s, *vs.* 11 ± 0.4 µV.s; $p<0.05$) and post-inspiration (15 ± 2 µV.s, *vs.* control: 33 ± 3 µV.s; $p<0.05$), and similar AbN activity (0.5 ± 0.2 bpm, *vs.* control: 0.3 ± 0.1 bpm) during normocapnia when compared to controls. Under hypercapnia, the PN bursts frequency decreased in *in situ* preparations of WD rats (Δ: -13 ± 6 %, *vs.* control: 10 ± 1 %; $p<0.05$), whereas PN (Δ: 24 ± 5 %, *vs.* control: 8 ± 1 %; $p<0.05$) and HN burst amplitudes (Δ: 71 ± 23 %, *vs.* control: 4 ± 1 %; $p<0.05$), cVN activity during inspiration (Δ: 9 ± 4 %, *vs.* control: -5 ± 1 %; $p<0.05$) and post-inspiration (Δ: 64 ± 30 %, *vs.* control: -10 ± 2 %; $p<0.05$), and AbN activity (Δ: 11060 ± 2015 %, *vs.* control: 2342 ± 937 %; $p<0.05$) were amplified. The results show that WD for 48 h modifies baseline respiratory activity *in situ*, and enhances reflex inspiratory, expiratory motor responses to hypercapnia.

Disclosures: **E.F. Silva:** None. **L.B. Nakai:** None. **J.V. Menani:** None. **D.S.A. Colombari:** None. **D.B. Zoccal:** None. **E. Colombari:** None.

Poster

231. Respiration: Modulation and Regulation

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 231.03/O23

Topic: E.08. Respiratory Regulation

Support: NIH/NIBIB U01EB021960
NIH/NCCIH R01AT008632
FAPESP 15/23568-8
FAPESP 13/17.251-6
CNPq 310331/2017-0

Title: Expiratory inhibition is a mechanism for adaptive control of breathing

Authors: *W. H. BARNETT[1], K. C. FLOR[2], M. K. AMARANTE[2], D. B. ZOCCAL[2], Y. I. MOLKOV[1];
[1]Dept. of Mathematics and Statistics, Georgia State Univ., Atlanta, GA; [2]Sao Paulo State Univ., Araraquara, Brazil

Abstract: There is accumulating evidence that expiratory neurons of the Bötzinger Complex (BC) provide a mechanism for adaptive control of breathing. Here we integrate new experimental data and mathematical modeling to gain new insights in the inhibitory connectome within the respiratory central pattern generator (rCPG). We infer inhibitory connectivity motifs by assimilating data on how differential manipulations of glycinergic and GABAergic inhibition

within the BC modulate the expression of active expiration (AE). Previously, we investigated the emergence of AE in hypercapnia, and we proposed that decrementing expiratory inhibition in the rCPG controlled the excitability of neurons of the parafacial respiratory group (pFRG) and, hence, their ability to fire action potentials during the late expiratory phase (late-E). Using our computational model, we showed that either increased chemosensitive drive to late-E neurons in the pFRG or a reduction in the firing rate in post-I neurons could facilitate AE. In this study, we applied specific $GABA_A$ or glycine receptor antagonists in the BC via microinjection with opposing effects of AE. The blockade of $GABA_A$ receptors in BC in normocapnia evoked AE. The blockade of glycine receptors suppressed AE during hypercapnia. In animals conditioned by sustained hypoxia (SH), AE occurs at normocapnia, and in these animals the blockade of glycine receptors also suppressed AE in normocapnia. These observations were reproduced in the extended computational model by discerning mixed glycinergic and GABAergic circuitry within the BC. In this model, late-E neurons of the pFRG received inhibition from a glycinergic post-I population and a GABAergic post-I population. The GABAergic post-I population inhibited a glycinergic augmenting expiratory (aug-E) population. The glycinergic aug-E population inhibited the glycinergic post-I population. The blockade of glycinergic receptors in the BC results in the disinhibition of the glycinergic post-I population. Its increased activity suppressed late-E activity in the pFRG regardless of whether it was evoked by hypercapnia or SH conditioning. The blockade of GABA receptors results in disinhibition of the glycinergic aug-E population from decrementing expiratory GABAergic inhibition. Increased aug-E activity suppresses the glycinergic post-I population, leading to the disinhibition of late-E neurons from expiratory inhibition. In summary, we suggested differential roles of glycinergic and GABAergic inhibition within the BC and extended our computational model to elaborate on glycinergic and GABAergic circuitries within the BC.

Disclosures: **W.H. Barnett:** None. **K.C. Flor:** None. **M.K. Amarante:** None. **D.B. Zoccal:** None. **Y.I. Molkov:** None.

Poster

231. Respiration: Modulation and Regulation

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 231.04/O24

Topic: E.08. Respiratory Regulation

Title: Delivery of morphine to mouse prefrontal cortex alters minute ventilation

Authors: *Z. T. GLOVAK[1], C. B. O'BRIEN[1], H. A. BAGHDOYAN[1,2,3], R. LYDIC[1,2,3]; [1]Psychology, [2]Anesthesiol., Univ. of Tennessee, Knoxville, TN; [3]Oak Ridge Natl. Lab., Oak Ridge, TN

Abstract: Mu opioid receptors have been localized within the rat prefrontal cortex (PFC) (*Trends Neurosci* 18: 22, 1995) yet, to our knowledge, the respiratory effects of opiates administered directly into mouse PFC have not been characterized. The present study is testing the two-tail hypothesis that microinjection of opiates into the PFC of freely behaving C57BL/6J (B6) mouse causes site-specific alterations in breathing. Adult male mice (n=8) were maintained on a 12:12 light:dark cycle in a thermoneutral environment with free access to food and water. Mice were anesthetized and implanted with a 26-gauge guide cannula aimed at the frontal association cortex (histology pending). After one week of recovery and one week of conditioning, awake mice received microinjections (50 nL) of saline (vehicle control) and morphine (3 nmol; 2.28 ng). All injections were performed within 2.5 to 3 h of light onset. Each mouse received three saline and three morphine microinjections in a randomized, counterbalanced, within-subjects design. The dependent measure of minute ventilation (VE) was quantified via whole body plethysmography as mL/min/g body weight for 1 h after saline or morphine administration. To avoid inflated degrees of freedom, the VE measures derived from 48 experiments were expressed as mean values for each mouse. Descriptive statistics showed that morphine caused divergent changes in VE. In one group of mice (n=4) VE (mean ±SD) was decreased 25% by morphine (1.93±0.22) when compared to saline (2.58±0.32). In a second group (n=4) VE increased by 39% from saline (2.22±0.16) to morphine (3.07±0.34). Paired t-tests revealed that the increase and decrease in VE were each statistically significant ($p<0.05$). These results support the interpretation that morphine delivered to the PFC of B6 mouse causes site-specific changes in VE. Although the PFC contains no respiratory neurons, the present results are consistent with human imaging data suggesting that breathing can be modulated by the PFC (*J Neurosci* 29: 8177, 2009) and with evidence that rodent breathing is altered by PFC drug delivery (*J Physiol* 591: 6069, 2013). Anterograde axonal labeling studies in rat have identified PFC projections to the dorsal motor nucleus of the vagus and to the nucleus of the solitary tract (*Brain Res Bull* 19: 639, 1987). Our ongoing studies are microinjecting fentanyl, buprenorphine, and naloxone, targeting additional regions of the mouse PFC, and increasing the number of mice studied.

Disclosures: **Z.T. Glovak:** None. **C.B. O'Brien:** None. **H.A. Baghdoyan:** None. **R. Lydic:** None.

Poster

231. Respiration: Modulation and Regulation

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 231.05/O25

Topic: E.08. Respiratory Regulation

Support: JSPS KAKENHI Grant 18K17783
JSPS KAKENHI Grant 17H05540

JSPS KAKENHI Grant 17K08559

Title: Dual orexin receptor antagonism and ventilatory function in mice

Authors: *I. FUKUSHI[1,2], S. YOKOTA[3], K. TAKEDA[4,1], J. TERADA[5,1], Y. OKADA[1]; [1]Clin. Res. Ctr., Murayama Med. Ctr., Musashimurayama, Japan; [2]Fac. of Hlth. Sci., Iryo Sosei Univ., Iwaki, Japan; [3]Dept. of Anat. and Neurosci., Shimane Univ., Izumo, Japan; [4]Fac. of Rehabilitation, Sch. of Healthcare, Fujita Hlth. Univ., Toyoake, Japan; [5]Dept. of Respirology, Grad. Sch. of Med., Chiba Univ., Chiba, Japan

Abstract: A dual orexin receptor (OX1R and OX2R) antagonist, suvorexant (Belsomra (R)), inhibits the activation of the arousal system in the central nervous system and facilitates the induction and maintenance of sleep. Recently, suvorexant has been widely used for treatment of insomnia. Because many of sleep-inducing drugs suppress ventilation, concerns could be raised whether suvorexant affects ventilation. However, the effects of suvorexant on the ventilatory control have not been fully studied. To address this issue, we first conducted immunohistological analysis of the orexin receptor (OX2R) expression in the brainstem respiratory regions with putative respiratory neuron markers:, i.e., Phox2b in the parafacial respiratory group/retrotrapezoid nucleus (pFRG/RTN), and NK1R/somatostatin in the preBötzinger complex (preBötC) of the mouse brainstem, respectively. Next, we analyzed ventilatory parameters as well as EEG in room air, hypercapnic (5% CO_2) and hypoxic (10% O_2) conditions after i.p. administration of saline and two doses (10 mg/kg and 100 mg/kg) of suvorexant. We measured respiratory flow by whole body plethysmography, and obtained tidal volume, respiratory rate, and minute ventilation. The carbon dioxide and oxygen concentrations in the chamber were monitored with a CO_2/O_2 gas analyzer, and were controlled by the amount of mixed gas flows into the chamber. Immunohistological analysis demonstrated that the OX2R is expressed in Phox2b-immunoreactive neurons and double-positive neurons for NK1R and somatostatin immunoreactivity in the pFRG/RTN and preBötC, respectively, suggesting the involvement of orexin in respiratory control. However, significant difference in ventilation was not observed before and after suvorexant administration. Suvorexant did not suppress either hypercapnic or hypoxic ventilatory response. Although the suvorexant doses (per body weight) in the present study were much higher than clinical doses, suvorexant did not affect ventilation in mice. Suvorexant may be safely used without suppressing ventilation in humans, which must be confirmed in clinical trials.

Disclosures: **I. Fukushi:** None. **S. Yokota:** None. **K. Takeda:** None. **J. Terada:** None. **Y. Okada:** None.

Poster

231. Respiration: Modulation and Regulation

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 231.06/O26

Topic: E.08. Respiratory Regulation

Support: FAPESP
CNPq
CAPES-PROEX
NIH/NHLBI
Dravet Foundation

Title: Cholinergic neurons in the pedunculopontine tegmental nucleus modulate breathing in rats by direct projections to the retrotrapezoid nucleus

Authors: *J. D. LIMA[1], C. R. SOBRINHO[1], L. K. SANTOS[1], B. FALQUETTO[2], A. T. TAKAKURA[3], D. K. MULKEY[4], T. S. MOREIRA[1];
[1]Dept. of Physiol. and Byophysics, [2]Dept. of Pharmacol., Univ. of Sao Paulo, Sao Paulo, Brazil; [3]Dept of Pharmacology, Inst. of Biomed. Science, Univ. of Sao Paulo, Sao Paulo, Brazil; [4]Dept. Physiol. and Neurobio., Univ. Connecticut, Storrs Manfld, CT

Abstract: The pedunculopontine tegmental nucleus (PPTg) in the mesopontine region has important physiological functions, including breathing control. The PPTg contains a variety of cell types, including cholinergic neurons that project to the rostral aspect of the ventrolateral medulla. In addition, cholinergic signaling in the retrotrapezoid nucleus (RTN), a region that contains neurons that regulate breathing in response to changes in CO2 /H+ , has been shown to activate chemosensitive neurons and increase inspiratory activity. Here, we aimed to identify the source of cholinergic input to the RTN and determine whether cholinergic signaling in this region influences baseline breathing or the ventilatory response to CO_2 in conscious male Wistar rats. Retrograde tracer Fluoro-Gold injected into the RTN labelled a subset of cholinergic PPTg neurons that presumably project directly to the chemosensitive region of the RTN. In unrestrained awake rats, unilateral injection of the glutamate (10 mm/100 nL) in the PPTg decreased tidal volume (VT) but otherwise increased respiratory rate (fR) and minute ventilation (VE). All respiratory responses elicited by PPTg stimulation were blunted by prior injection of methyl-atropine (5 mm/50 nL) into the RTN. These results show that stimulation of the PPTg can increase respiratory activity in part by cholinergic activation of chemosensitive elements of the RTN. Considering cholinergic PPTg projections also activated expiratory drive from the parafacial respiratory group, we speculate that simultaneous activation of both regions will favor increased respiratory frequency but at the expense of limited tidal volume.

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Poster

231. Respiration: Modulation and Regulation

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 231.07/O27

Topic: E.08. Respiratory Regulation

Support: NIH Grant K99 HL145004
NIH Grant F32 HL134207
NIH Grant R01 HL126523

Title: Cell-type specific effects of substance P facilitate recurrent excitation to increase breathing frequency

Authors: *N. A. BAERTSCH, J.-M. RAMIREZ;
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Abstract: Breathing frequency is largely determined by expiratory time (TE), the time between inspiratory breaths. Inspiration originates from the preBötzinger Complex (preBötC), a network of excitatory and inhibitory neurons in the medulla. Three phases of activity can be discriminated within the preBötC. Following each inspiratory burst (TI), cellular- and network-level mechanisms result in a refractory period (RP), which is positively related to the amount of synchronization among excitatory neurons, and negatively related to the amount of concurrent inhibition during the preceding burst. As the network becomes less refractory, mechanisms of recurrent excitation (RE) begin to build up within the network until the next inspiratory burst is generated. Thus, the duration of the RP and the rate of RE are important determinants of breathing frequency: TE=(RP+RE). We tested the hypothesis that neuromodulators regulate breathing frequency by differentially modulating TI, RP and RE through cell-type specific effects. Using brainstem slices from neonatal mice, we specifically tested the modulatory effects of Substance P (SP) since it is known to potently drive breathing frequency. To measure the RP, excitatory neurons were optogenetically stimulated with brief light pulses, and the probability of evoking a burst was quantified in relation to elapsed time following the preceding spontaneous burst. We found that the RP does not change in response to SP (0.5-1.0μM). Next, we combined optogenetic and intracellular recording approaches to define inspiratory neurons as excitatory or inhibitory and characterize changes in their firing patterns in response to SP. Excitatory neurons with spiking activity that ramps up during TE culminating in an inspiratory burst, known as pre-inspiratory neurons, are thought to constitute the primary drivers of RE. In response to SP, the spiking activity of these pre-inspiratory neurons was increased during TE but did not change during inspiratory bursts (TI). Excitatory neurons active during inspiratory bursts but silent

during TE either acquired pre-inspiratory spiking activity with no change in activity during TI, or did not change spiking activity during TE or TI. Inhibitory neurons active during inspiration were silent during TE under baseline conditions and in SP exhibited no change in spiking during TE or TI. Collectively, these results suggest that SP facilitates the amount of pre-inspiratory spiking among excitatory neurons during TE without altering spiking activity of excitatory and inhibitory neurons during TI, thus leaving the resulting RP unchanged. We conclude that SP modulates breathing by controlling the rate of RE.

Disclosures: **N.A. Baertsch:** None. **J. Ramirez:** None.

Poster

231. Respiration: Modulation and Regulation

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 231.08/O28

Topic: E.08. Respiratory Regulation

Title: Modulation of respiratory networks by substance P in the metamorphosing frog

Authors: *D. COMBES, J. SIMMERS, M.-J. CABIROL;
Univ. of Bordeaux, CNRS UMR 5287, BORDEAUX, France

Abstract: Frog metamorphosis involves a complete change of respiratory mode from aquatic to aerial breathing. At critical stages, functional larval and adult respiratory systems co-exist in the same animal, implying a progressive reconfiguration of underlying neural circuitries. Isolated *in vitro*, the brainstem of the clawed toad *Xenopus laevis* produces spontaneous respiratory output consisting of stage-specific patterns of bilaterally-synchronous impulse bursts in cranial motor nerves. In early premetamorphic tadpoles, high frequency, low intensity bursts corresponding to fictive gill ventilation are continuously generated by the brainstem. Episodic waves during which the intensity of those bursts increases are observed in later premetamorphic stages. In postmetamorphic froglets, short and high intensity bursts are produced at lower frequency, corresponding to fictive lung breathing. Pulmonary bursting appears at prometamorphic stages and becomes increasingly present through the course of metamorphosis.
The endogenous neuropeptide substance P (SP) is an important neuromodulator of rhombencephalic central pattern generating networks that control respiration in vertebrates. Here we study the modulatory and putative developmental role of SP during metamorphosis when the animal switches from aquatic (gill) to aerial (lung) breathing. Extracellular recording of appropriate cranial nerves of *in vitro* isolated brainstems is used to study the effect of SP on the rhythmic motor output of respiratory networks in both pre- and postmetamorphic animals. We show that SP exerts a dose-dependent increase of lung breath frequency in adults while decreasing the gill bursting amplitude in tadpoles, in both cases by acting via NK-1 receptors. Additionally, SP is able to switch-on lung respiration in premetamorphic tadpoles thereby

revealing the presence of underlying respiratory centers at this stage where only gill respiration is effective. Immunostaining revealed NK-1 receptors located on respiratory motoneurons' membrane in facial and vagal nuclei. We conclude that SP should play a role in triggering the onset of spontaneous lung breathing, thereby potentially participating to the maturation of the network in developing tadpoles.

Disclosures: D. Combes: None. **J. Simmers:** None. **M. Cabirol:** None.

Poster

231. Respiration: Modulation and Regulation

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 231.09/O29

Topic: E.08. Respiratory Regulation

Support: NIH Grant NS110169
Craig H Neilsen 546714

Title: Functional multiplexing of airway behaviors along the ventral respiratory column in ventrolateral medulla

Authors: *N. M. MELLEN[1], T. PITTS[2], A. HUFF[2], M. M. REED[2];
[2]Neurolog. Surgery, [1]Univ. of Louisville, Louisville, KY

Abstract: In rat, networks along the ventral respiratory column maintain blood-gas homeostasis via respiration, but these same networks mediate thermoregulation, exploratory behavior (sniffing/whisking), and ingestion, among others. These diverse behaviors are precisely coordinated with breathing via afferent feedback from lungs and as-yet unspecified propriobulbar interneuronal networks that modulate respiration on a cycle-to-cycle basis. Here, anatomically discrete loci for lung afferent feedback and swallow in dorsomedial medulla, identified by others *in vivo*, are exposed at the dorsal edge of the sagittally-sectioned rat hindbrain preparation (SSRH) for activation via bipolar (Pt:Ir, MicroProbes) electrode. Simultaneous recordings from hypoglossal nerve rootlet (XIIn) and ventral root C2, enabled identification of the system-level correlates of swallow (identified as a burst at XIIn but not C2), and the Breuer-Hering (BH) reflex (shortened I and lengthened E in response to stimuli during I and E respectively), elicited via stimulation (20 Hz and 10 Hz, 8 V; S88 84T50D, Grass Instruments). In addition, by incubating the SSRH in the synthetic Ca^{2+} indicator Cal520 (K_D=0.32 μM, AAT labs), simultaneous high-speed (50 Hz) optical recording were obtained with a large-format sCMOS camera (Prime 95B, Photometrics). Optical recordings delineated how respiration-modulated networks in ventrolateral medulla were reconfigured during swallow, or during the BH reflex. In addition, novel, spatially compact networks were identified rostral and dorsal to the facial nucleus (VIIn), and at the dorsal margin of VIIn, that were activated during

fictive swallow. Together these findings establish proof-of-concept for the feasibility of investigating *in vitro*, how functionally heterogeneous networks along the VRC interact and reconfigure to enable this larger repertoire of behaviors.

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Poster

231. Respiration: Modulation and Regulation

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 231.10/O30

Topic: E.08. Respiratory Regulation

Support: FAPESP grant 2013/10484-5
FAPESP grant 2016/24994-3

Title: Active expiratory oscillator regulates nasofacial and oral motor actions in rats

Authors: *D. J. MORAES[1], M. DA SILVA[2], A. DE BRITTO[2];
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Abstract: Active expiration is mediated by expiratory oscillator located in the parafacial Respiratory Group (pFRG). Actively expiring requires more than contract expiratory muscles; i.e. multiple cranial nerves should be recruited to stabilise the naso- and oropharynx and airways. We tested the hypothesis that pFRG activation recruits Facial and Trigeminal motoneurons to control, respectively, naso- and oropharyngeal resistance of rats. A combination of electrophysiology and pharmacology allowed us to identify brainstem circuitries that phase-locking active expiration, nasofacial and oral motor actions in *in situ* preparation of rats. High chemical drive (hypercapnia/acidosis) or pFRG unilateral excitation (glutamate injection) evoked active expiration and simultaneous discharges in the motoneurons (facial: 55.1 ± 3.07 vs 4.1 ± 0.55 Hz; $p<0.05$; n=6) (trigeminal: 76.16 ± 2.05 vs 4.28 ± 0.98 Hz; $p<0.05$; n=5)] and motor nerves responsible for the control of nasofacial [(buccal nerve: 0.43 ± 0.02 vs 0.16 ± 0.01 s; $p<0.05$; n=16) (zygomatic nerve: 0.43 ± 0.01 vs 0.18 ± 0.02 s; $p<0.05$; n=16)] and oral (mylohyoid nerve: 0.65 ± 0.04 vs 0.19 ± 0.02 s; $p<0.05$; n=14) motor actions. Bilateral pharmacological inhibition (GABAergic and glycinergic receptors activation) of pFRG abolished active expiration and the expiratory nasofacial [(buccal nerve: 0.22 ± 0.05 vs 0.43 ± 0.02 s; $p<0.05$; n=6) (zygomatic nerve: 0.21 ± 0.05 vs 0.43 ± 0.01 s; $p<0.05$; n=6)] and oral (mylohyoid nerve: 0.2 ± 0.02 vs 0.65 ± 0.04 s; $p<0.005$; n=5) motor responses evoked by hypercapnia/acidosis. We conclude that pFRG provides the excitatory drive to phase-locking

rhythmic nasofacial and oral motor actions with expiration in rats, which should be predominant responses during exercise, anxiogenic behaviours or during airways obstruction.

Disclosures: D.J. Moraes: None. **M. da Silva:** None. **A. de Britto:** None.

Poster

231. Respiration: Modulation and Regulation

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 231.11/O31

Topic: E.08. Respiratory Regulation

Support: NIH/NINDS U01-NS0904143 Center for SUDEP Research

Title: The central amygdala (CeA) plays a key role in ventilatory arrest and SUDEP in a Dravet syndrome mouse model (DS)

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Abstract: RATIONALE: Sudden unexpected death in epilepsy (SUDEP) is the most common cause of death in refractory epilepsy patients. The MORTEMUS study showed that SUDEP patients displayed severe postictal respiratory and cardiac dysfunction. However, the mechanisms involved in causing these changes are unknown. Recent research has implicated the amygdala in ventilatory arrest. Seizures that spread into the amygdala cause central apnea with concomitant O_2 desaturation. This can also be elicited in patients by stimulating the amygdala with depth electrodes without inducing seizures. Here, we examined the role of the amygdala in peri-ictal respiratory arrest in a DS mouse model (*Scn1a*$^{R1407x/+}$). **METHODS**: Electrolytic lesions were performed in the CeA of DS mice, after which they were instrumented with a headmount to collect EEG, ECG and EMG data. DS mice were placed in a plethysmography chamber and seizures were induced by using a heat lamp to raise body temperature from 36 to 42.5 C or until a seizure with full hindlimb extension and ventilatory arrest occurred. The locations of lesions were verified histologically. Electrical stimulation of the CeA was performed with a monopolar electrode while animals were lightly anesthetized with isoflurane 0.5 - 1%. Baseline breathing, the hypercapnic ventilatory response (HCVR) and the hypoxic ventilatory response (HVR) were measured in DS mice before and after CeA lesions using whole-body plethysmography. Baseline breathing was measured with head-out plethysmography during CeA stimulation. **RESULTS**: CeA lesions did not affect baseline breathing, HCVR or HVR in DS mice. CeA lesions also did not prevent heat-induced seizures. Preliminary data showed that respiratory arrest and death occurred in control mice after heat-induced seizures in 58.8% (10/17)

of cases. In contrast, only 9.1% (1/11) of mice showed ventilatory arrest and death following CeA lesions (p=0.0161). CeA stimulation caused apnea that was dependent on the frequency and amplitude of stimulation, without a seizure. At 50 Hz and 500 micro Amp apnea could be induced for as long as 4 minutes without causing death, and without reversal of anesthetic immobility. **CONCLUSIONS**: These results indicate that the amygdala is not involved in normal breathing or CO_2 chemoreception, as ablation did not affect baseline breathing or the HCVR. However, CeA stimulation induced prolonged central apneas whereas there was a trend for CeA ablation to prevent ventilatory arrest and death in DS mice. Our findings suggest that the amygdala may be an important component of the neural pathway involved in seizure spread from the forebrain to brainstem that leads to ventilatory arrest and SUDEP.

Disclosures: E. Bravo: None. **A. Marincovich:** None. **Y. Kim:** None. **M.S. Crotts:** None. **F.A. Teran:** None. **B.J. Dlouhy:** None. **G.B. Richerson:** None.

Poster

231. Respiration: Modulation and Regulation

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 231.12/O32

Topic: E.08. Respiratory Regulation

Support: CNRS and ANR-15-CE16-0013

Title: Phox2b expression defines premotor neurons in the intermediate reticular formation involved in oro-motor behavior

Authors: B. DEMPSEY, *G. FORTIN;
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Abstract: Orofacial behaviours like licking, chewing and swallowing require the orchestration of diverse motor effectors (8 within tongue alone!) whilst underlying autonomous rhythms (i.e. breathing) are respected and the capacity for rapid adaption to interoceptive or environmental stimuli is retained. In this study, we map premotor neurons for hypoglossal and branchiomotor neurons. Monosynaptic retrograde tracing from the posterior digastric, the geniohyoid and genioglossus muscles (the two former of which are involved in the initiation of jaw opening and the latter of which, tongue protrusion) resulted in dense labelling of premotor neurons within the intermediate reticular formation (IRt) that we find originating from *Olig3*-expressing progenitors, and expressing *Phox2b*, *Lmx1b*, and *Tlx3*. Anterograde tracing from *Phox2b*-IRt neurons with viral vectors in the adult mouse revealed predominant outputs to oro-motor targets, notably the peri-trigeminal area, the medial portion of the facial nucleus, the hypoglossal nucleus, the contralateral IRt, and a descending projection to the cervical spinal cord. Conversely, monosynaptic retrograde tracing from Phox2b-IRt neurons revealed distant inputs

from the motor cortex, deep superior colliculus, interposed cerebellar nuclei, trigeminal mescencephalic nucleus and peri-trigeminal area, as well as local inputs from the surrounding reticular formation and the NTS. Neither anterograde nor retrograde tracing from Phox2b-IRt neurons revealed monosynaptic communication with the ventral respiratory column, unexpectedly given the requirement for oromotor/respiratory coordination. We have thus genetically identified a major population of premotor neurons controlling oro-facial muscles.

Disclosures: B. Dempsey: None. **G. Fortin:** None.

Poster

231. Respiration: Modulation and Regulation

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 231.13/O33

Topic: E.08. Respiratory Regulation

Support: RIKEN Healthcare and Medical Data Platform Project

Title: The properties of the neurons considered the coordination of nociception-respiration in the parabrachial nucleus

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Abstract: The sensation of nociceptive signals projects to the lateral parabrachial nucleus (LPB) of the pons via the dorsal horn; and LPB has also known as the system of inspiratory-expiratory (I-E) phase switching that contributes to the control of respiratory rate. The tight interaction between respiration and pain signals as nociception-respiration coordination were expected in LPB. In this study, we investigated the nociceptive-respiratory system using the pons-medulla-spinal cord preparation intact forelimb isolated from postnatal 0-2 days-old-rats. Respiratory activity was recorded from cervical fourth (C4) ventral nerve root. The C4 inspiratory rate increased significantly when a small amount of 2% capsaicin was injected into forelimb with pons, but the removal of pons had no effects. Moreover, C4 inspiratory rate also increased significantly with C8 dorsal root stimulation as a noxious stimulation. We examined the responded area of LPB stimulated by C8 dorsal root using optical imaging with voltage-sensitive dye, and the LPB neurons were recorded from the responded area using whole-cell patch-clamp. I-E neurons which were synchronized with the Inspiratory-Expiratory phase of C4 activity existed in the LPB and KF. The spontaneous firing neurons which were not synchronized with the C4 activity were called non-respiratory neurons and existed in the LPB. In the external LPB, all I-E neurons and the half of recorded non-respiratory neurons were responded by C8 dorsal

root stimulation. Non-respiratory neurons in the superficial layer of LPB received C8 dorsal root stimulation and showed a post-inhibitory rebound (PIR) caused by hyperpolarizing current pulse application. Non-respiratory neurons in the external LPB received the noxious sensory input and facilitated respiratory network; besides, the firing pattern of I-E neurons in the external LPB might change from inspiratory neuron to I-E neuron activated by non-respiratory inputs. These results suggested that I-E neurons could receive noxious information, so I-E neurons were thought to be the core mechanism of nociceptive-respiratory coordination; the non-respiratory LPB neurons which expressed PIR might be participating in the onset mechanism of the nociceptive-respiratory relay network.

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Poster

231. Respiration: Modulation and Regulation

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 231.14/O34

Topic: E.08. Respiratory Regulation

Support: NIH Grant R01-NS086088

Title: Adaptive respiratory pacing restores ventilatory function in incomplete spinal cord injured rats

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Abstract: Trauma, disorder, or disease of any of the components that comprise the ventilatory control loop may impair ventilatory capabilities in a manner that can result in hypoventilation. While mechanical ventilation is the standard for ventilatory support, there is risk of inducing alveolar damage and causing diaphragm atrophy. Respiratory pacing circumvents these risks by producing ventilation through electrical stimulation of the diaphragm muscle or phrenic nerve. However, commercially-available ventilatory pacing systems are all open-loop, leading to inability to adapt to changes in metabolic demands and other changes that may affect effectiveness of pre-set stimulation parameters. We have addressed this by developing a biologically-inspired closed-loop adaptive controller for respiratory pacing that is capable of eliciting a ventilatory response akin to the intrinsic ventilatory response to CO_2.
The adaptive controller uses a neuromorphic pattern generator (PG) pattern shaper (PS) structure. The PG, based on a biological respiratory central pattern generator model, responds to end-tidal CO_2 ($etCO_2$) by determining the proper ventilatory response. The PS then compares the ventilatory output (breath volume) to the desired ventilatory pattern to determine, through an

artificial neural network, the timing and amplitude of stimulation required to elicit the pattern dictated by the PG. The synergy between these two modules allows the controller to respond to metabolic-induced changes in $etCO_2$ and to biomechanical and stimulation-related changes that might affect pacing efficiency.
Acute studies in an animal model of incomplete spinal cord injury were performed to test the PG/PS controller's ability to respond to $etCO_2$ and control ventilation. Spinal cord-injured Sprague-Dawley rats (n = 2) were anesthetized via urethane and tracheotomized to measure pulmonary flow, which was then integrated to obtain breath volume. A capnograph sampled exhaled air to obtain $etCO_2$. Stimulating electrodes were implanted bilaterally on the diaphragm muscle and then connected to a programmable stimulator controlled by the PG/PS controller. Spinal injury was induced by hemisection of the spinal cord at the C2 level and verified through EMG recordings of the ipsilateral hemidiaphragm. Results showed that the controller was able to automatically adjust ventilation to reduce $etCO_2$ levels from a hypercapnic range of 6.63 ± 0.25 towards a normocapnic range of 5.58 ± 0.31. While additional pre-clinical studies need to be performed, these results suggest that the PG/PS controller can restore ventilatory function after spinal cord injury.

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Poster

231. Respiration: Modulation and Regulation

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 231.15/O35

Topic: E.08. Respiratory Regulation

Support: NS085226
HL105511
HL142752
Wisconsin Alumni Research Foundation UW2020 grant

Title: Automated detection of apneas and hypopneas from rodent plethysmography

Authors: *J. A. PFAMMATTER[1], J. N. OUELLETTE[3], J. G. WELTMAN[3], M. V. JONES[2], T. BAKER-HERMAN[3], J. J. WATTERS[4];
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Abstract: We present an algorithm for the unbiased, fully automated detection of apneas and hypopneas from rodent plethysmography records. The software first identifies all events (breaths, animal movement, and artifact) within a filtered (high-pass forward/reverse at 1 Hz) and normalized (mean/std) recording using a 'two-threshold' method where an event is first

defined as signal clips between a high threshold and low threshold equal to 40% and 10% of the standard deviation of the signal, respectively. Then, the start and end of each event are adjusted by finding the nearest zero crossing of the first derivative of the signal prior to the initial start and after the initial end of the event. Then, the interval between breaths of 'normal' breathing periods is probabilistically identified (Gaussian fitting) using amplitude and inter-event interval characteristics. Apneas were defined as >2 missed breaths, and hypopneas were defined as <60% of normal breath tidal volume. To validate the algorithm, we compared the automated and manual scoring (1-2 humans per record) of apneas in Sprague Dawley rats treated in normoxia (Nx) or chronic intermittent hypoxia (CIH) conditions. Rats were exposed to 7 days of CIH (2 min 10.5%/21% FiO2, 8 hrs) or normoxia, and ventilation was recorded using plethysmography before, 1 day after, and 7 days after CIH. CIH is expected to result in an increased frequency of apneas/hypopneas during sleep, an effect that progressively and spontaneously recovers following CIH termination via unknown mechanisms. While a full comparison between the algorithm and human scoring is forthcoming, preliminary results (15 records with 2 humans scoring each) indicate that the computer found ~88% of the apneas identified by humans. Automated scoring indicated that animals treated with CIH had ~ 7 more apneas per hour compared to pre-CIH treatment whereas animals treated with Nx no increase in apnea frequency. This algorithm scores records quickly (6-hour record in ~3 minutes), doesn't require parameter selection or training, is adaptive to individual animal baseline breathing variability, and automatically differentiates between post-sigh and spontaneous apneas.

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Poster

231. Respiration: Modulation and Regulation

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 231.16/O36

Topic: E.08. Respiratory Regulation

Support: Research reported in this publication was supported by the National Heart Lung and Blood Institute (1R15HL126105) and National Institute of General Medical Sciences (1SC2GM112570) of the National Institutes of Health, an Institutional Development Awa

Title: Assessing lung priming burst characteristics and CO_2 sensitivity in bullfrog tadpole brainstems across metamorphosis

Authors: M. G. CARMEL[1], I. MAJEWSKA[1], M. D. REED[2,3], M. B. HARRIS[4,2], B. E. TAYLOR[4,2], ***K. E. ICEMAN**[1,2];
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Fairbanks, AK; [3]Dept. of Neurolog. Surgery, Univ. of Louisville, Louisville, KY; [4]Dept. of Biol. Sci., California State Univ. Long Beach, Long Beach, CA

Abstract: Bullfrog tadpoles ventilate both the buccal cavity and lung. Air must be forced into the lungs by elevation of the buccal floor, which compresses the buccal cavity; this action has been termed the "lung powerstroke". To prepare for this lung inflation, the buccal floor must first be depressed to expand the buccal cavity as a priming action. This priming buccal expansion immediately precedes the lung powerstroke. The centrally-generated burst patterns associated with the priming buccal expansion and lung powerstroke have been recorded from isolated brainstems and recently described. The region controlling priming burst patterns is thought to reside in the rostral medulla, surrounding the lung oscillator. In the current study, we measure activity of facial and hypoglossal nerves in brainstems isolated from tadpoles at early and late developmental stages, under normal and elevated levels of CO_2. We have recently shown that the midbrain/pons influences CO_2 responsiveness and timing of both buccal and lung ventilatory bursting, depending on the stage of larval development. Here, brainstems are either left relatively intact or transected to remove the midbrain/pons in order to determine the influence of this region on priming burst patterns. We describe properties of priming bursts relative to subsequent lung powerstrokes. We compare these properties between larval stages and assess CO_2 response and the effect of midbrain/pons transection. These results contribute to the relatively recent effort to characterize the priming phase of tadpole lung ventilation.

Disclosures: M.G. Carmel: None. **I. Majewska:** None. **M.D. Reed:** None. **M.B. Harris:** None. **B.E. Taylor:** None. **K.E. Iceman:** None.

Poster

231. Respiration: Modulation and Regulation

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 231.17/O37

Topic: E.08. Respiratory Regulation

Title: The effects of acute and chronic intermittent hypoxia on sympathetic tone and body weight regulation in mice

Authors: *H. R. PETERSON, S. N. FRAMNES-DEBOER, D. M. ARBLE;
Dept. of Biol. Sci., Marquette Univ., Milwaukee, WI

Abstract: Numerous studies have described a relationship between obstructive sleep apnea, characterized by intermittent hypoxia (IH), and an increase in sympathetic activation. However, it is unclear whether IH leads to an immediate increase in sympathetic tone or if chronic exposure is necessary. This increase in sympathetic tone has been hypothesized as a key mechanism linking IH to cardiometabolic disease, including obesity and diabetes. To determine

the relative timeframe of sympathetic activation and its association with obesity and diabetes, male C57bl/6J mice maintained on a high fat diet were exposed to IH for 9 hr/day both acutely (1 day) and chronically (6 days). Norepinephrine (NE) concentration, a key neurotransmitter associated with sympathetic tone, was analyzed following IH exposure using urine analysis. Body weight, food intake, and glucose tolerance were accessed before and after IH exposure. We found that a single 9 hr exposure was not sufficient to increase NE levels. However, acute IH treatment was sufficient to increase glucose tolerance, implicating that IH-induced alterations in glucose tolerance can occur independent of sympathetic tone. Chronic (6 day) exposure to IH mice led to a significant increase in urine NE levels. Similar to the acute treatment, chronic IH also led to an increase in glucose tolerance. Unlike the acute exposure, chronic IH exposure mitigated weight gain in high-fat diet fed mice. These data support the conclusion that IH-induced increases in sympathetic tone are associated with negative energy balance. Taken together, these results demonstrate that 6 days of IH is sufficient to increase NE levels in mice and that an IH-induced rise in sympathetic tone may be responsible for weight loss in mice. Further studies in humans will provide insight into elevated sympathetic tone and its involvement in cardiometabolic disease.

Disclosures: H.R. Peterson: None. **S.N. Framnes-DeBoer:** None. **D.M. Arble:** None.

Poster

231. Respiration: Modulation and Regulation

Location: Hall A

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Program #/Poster #: 231.18/O38

Topic: E.08. Respiratory Regulation

Support: NS085226
HL105511
HL142752
Wisconsin Alumni Research Foundation UW2020 grant

Title: Gestational intermittent hypoxia sex-dependently impairs the neural control of breathing and alters the microglial transcriptome in adult offspring

Authors: J. J. WATTERS, A. L. MEZA, M. G. GUMNIT, C. R. MICKELSON, A. C. EWALD, E. A. KIERNAN, J. N. OUELLETTE, ***T. L. BAKER**;
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Abstract: The *in utero* environment is well-known to influence CNS development and microglial function, effects that can last into adulthood. However, fetal brain reprogramming has never been evaluated in the context of sleep-disordered breathing (SDB) during pregnancy, despite its alarming increase in recent years and detrimental consequences to the newborn. Here,

we tested the hypothesis that maternal exposure to intermittent hypoxia during pregnancy alters the neural control of breathing and the microglial transcriptome in CNS regions critical for respiratory neural control of the adult offspring. Rat dams were exposed to chronic intermittent hypoxia (8 hrs/day, 2 min 10.5% O2 separated by 2 min of 21% O2) or intermittent normoxia from gestation days 10-21 (GIH and GNX, respectively). The frequency of spontaneous apneas and neural responses to recurrent reductions in respiratory neural activity were assessed in male and female offspring (8-12 wks old). Male, but not female, GIH offspring exhibited significantly more spontaneous apneas during presumptive sleep than their control GNX counterparts. Similarly, compensatory neural responses to recurrent central apneas (without hypoxia) were impaired in male, but not female, GIH offspring. To determine if microglia underlie impaired compensatory plasticity in GIH offspring, microglia were depleted with Pexidartinib (PLX3397; 80 mg/kg, 7 days po), a colony stimulating factor 1 receptor (CSFR1) inhibitor that results in microglial apoptosis. PLX3397 significantly reduced Iba1+ and CD11b+ cells throughout the brain and spinal cord and restored the capacity for recurrent central apneas to elicit compensatory plasticity in male GIH offspring, without affect in female GIH or GNX offspring of either sex. Microglia were immunomagnetically isolated from adult brainstem and cervical spinal cord, and total RNA harvested. RNA-Seq analyses indicated strong differences in the normal transcriptomes of GNX brainstem and cervical spinal cord microglia. Surprisingly, GIH potently altered gene expression in female spinal cord microglia, with little effect in males. Brainstem microglial gene expression was unaltered by GIH treatment in either sex. Together, these data suggest that maternal exposure to intermittent hypoxia impacts respiratory and microglial function in adult offspring.

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Poster

231. Respiration: Modulation and Regulation

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 231.19/O39

Topic: E.08. Respiratory Regulation

Title: Chemosensitivity of acutely dissociate serotonergic raphe neurons

Authors: Y. WU, E. BRAVO, *G. B. RICHERSON;
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Abstract: Rationale: Serotonergic neurons of the medullary raphe are putative respiratory chemoreceptors that detect changes in CO2/tissue pH. We hypothesized that they maintain their chemosensitivity after acute dissociation.Methods: The midline medulla was dissected from P10-P18 mice expressing YFP under control of the enhancer region of Pet1, which is selective for

serotonergic neurons. Tissue was enzymatically digested and triturated, and plated onto glass coverslips. Cells were fed with culture medium and allowed to attach to the substrate for 2-3 hours. Patch clamp recordings were then made in current clamp mode either immediately or after 2-4 days at which time neurons had regrown their major dendrites. In some cases, glial growth was inhibited by cytosine arabinoside. After recordings, coverslips were fixed with 4% formalin and immunostained with antibodies for MAP2 or GFAP, and it was determined whether any synaptic or glial contacts were present on recorded neurons.
Results: A subset of serotonergic neurons maintained a high degree of chemosensitivity after acute dissociation, with a 2-3 fold increase in firing rate from a baseline firing rate near 1 Hz in response to a decrease in pH from 7.4 to 7.2. This response is similar to that reported for these neurons in acute brain slices and in cell culture. Chemosensitivity was present even when there were no contacts made from other neurons or glia. The response to pH increased with time in culture consistent with a contribution from dendrites.Conclusions: Serotonergic neurons have a high degree of intrinsic (cell-autonomous) chemosensitivity, part of which may arise from dendrites.

Disclosures: Y. Wu: None. **E. Bravo:** None. **G.B. Richerson:** None.

Poster

231. Respiration: Modulation and Regulation

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 231.20/O40

Topic: E.08. Respiratory Regulation

Title: Prenatal hypoxia modulates the influence of $GABA_A$ and glycine receptors on fictive breathing in newborn rat

Authors: C. CARAVAGNA[1], A. R. CASCIATO[2], J. O. COQ[1], S. LIABEUF[1], C. BROCARD[1], L. BODINEAU[2], J. PEYRONNET[1], ***F. CAYETANOT**[2];
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Abstract: *Introduction:* The development of mammal neural system is influenced by early life experiences. According to the hypothesis of ‘‘developmental programming of health and disease’’ or ‘‘foetal origins of disease later in life’’, maternal environment, in which foetus develops, has an impact on its development, and even on adult health. Intra-uterine growth restriction related to prenatal hypoxia is recognized as an important risk factor for early postnatal respiratory illness. This prenatal hypoxia could disrupt the central respiratory drive and could influence the maturation of neurotransmitter systems. Our study aim at determining how prenatal hypoxia, affects the control of breathing and the functional development of the influence of $GABA_A$ and glycine receptors on breathing.

Methods: To determine the impact of prenatal hypoxia on central respiratory drive, pharmacological studies were coupled to electrophysiological recordings on *ex vivo* preparations from newborn rats (P0-1; P3-4) containing or not the pons (medullary spinal cord or ponto-medullary preparations) exposed to prenatal hypoxia (Hx) or not. We record fictive breathing frequency (f_R) at the level of C4 ventral roots (consist of phrenic axons). To complete the electrophysiological study, we quantified Cl^- co-transporters (KCC2) expression by Western blot, and BDNF content by Elisa test in pons.
Results: We showed that prenatal hypoxia significantly modified the influence of GABA and glycine on fictive f_R in *ex vivo* preparations from newborn rats. When the pons was present, the influence of picrotoxine (*p=0.046*) or strychnine *(p= 0.025)* differed at P3-4 between the two groups of pups. It seems related to change in functionality of Cl^- co-transporters induced by prenatal hypoxia. In the pons, at P0-1, KCC2 expression was similar in both groups. At P3-4, KCC2 expression was significantly decreased in Hx group in comparison with normoxic pups *(U=3; P=0.008)* in the pons. We noted a significant increase in BDNF content *(U=3.25; p=0.022)* in the pons in Hx pups in comparison with normoxic pups at P3-4.
Conclusions: Prenatal hypoxia leads to change in fictive breathing recorded in *ex vivo* preparations underpinned by central respiratory command perturbation. The blockade of $GABA_A$ or glycine receptors have different impacts on fictive breathing frequency in Hx pups compare to normoxic pups. A modification of Cl^- co-transporters expressions in the Hx pups, seems to be responsible for the fictive breathing response change to $GABA_A$ or glycine blockade. The decrease in KCC2 expression could be related to an increase in BDNF content.

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Poster

231. Respiration: Modulation and Regulation

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Topic: E.08. Respiratory Regulation

Support: French National Agency for Research (ProgestVentil; ANR-15-CE17)
Chancellerie des Universités de Paris (Legs Poix 1504)
Association Française pour le syndrome d'Ondine

Title: Improvement of hypoventilation in Phox2b mutant mice modelling the congenital central hypoventilation syndrome by etonogestrel, a potent progestin. Perspectives for a possible potentiation by serotoninergic drugs

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Abstract: Introduction: Congenital central hypoventilation syndrome (CCHS) is a life-threatening disorder characterized by hypoventilation during sleep and absence of the ventilatory response to CO_2/H^+. The disease-causing mutations are generally polyalanine repeat expansion mutations of *PHOX2B*. Knock-in mice bearing the 7-alanine expanded allele (i.e. 27Ala) of *PHOX2B* (the most frequent mutation in patients) exhibit massive loss of retrotrapezoid nucleus neurons, lack CO_2/H^+chemosensitivity, and die within hours after birth. No pharmacological treatment is available. Clinical observations revealed a recovery of the CO_2/H^+ chemosensitivity in two adult female patients with CCHS, both under desogestrel, a potent progestin used for contraceptive purpose. We previously showed that in mice, etonogestrel, the active metabolite of desogestrel, increased baseline respiratory frequency (f_R) by a medullary action involving the serotoninergic (5-HT) systems. Accordingly, we hypothesized that etonogestrel may stimulate f_R on *Phox2b* mutant mice modelling CCHS and that 5-HT drugs would improve this respiratory effect of etonogestrel. **Materials and Methods:** Experiments were made on *ex vivo* medullary-spinal cord preparations from conditional RTN/pFRG *Phox2b* mutant ($Egr2^{cre/+};Phox2b^{27Alaki}$) and WT newborn mice (P0-P2). f_R was assessed by recording the 4th spinal ventral root; it contains axons of phrenic motoneurons innervating the inspiratory muscle diaphragm. Effect of etonogestrel on f_R under normopH and metabolic acidosis conditions (modelling CO_2/H^+ *in vivo* conditions) was evaluated in presence or not of 5-HT drugs *i.e*. 5-HT reuptake inhibitor and 5-HT receptor agonists. **Results:** Without etonogestrel, the increase in f_R induced by metabolic acidosis observed in WT mice was absent in $Egr2^{cre/+};Phox2b^{27Alaki}$ mutant mice. Etonogestrel restored a respiratory response to metabolic acidosis in $Egr2^{cre/+};Phox2b^{27Alaki}$ mutant mice. In WT mice, when etonogestrel was associated with 5-HT reuptake inhibitor, the increase in f_R induced by metabolic acidosis was enhanced. Surprisingly, this potentiation was not retrieved in $Egr2^{cre/+};Phox2b^{27Alaki}$ mutant mice at the concentrations used in WT. **Conclusion:** Etonogestrel was able to alleviate the hypoventilation in *Phox2b* mutant mice modelling CCHS by a medullary action. Second, our data confirm that the modulation of 5-HT systems may improve the respiratory effect of etonogestrel in WT mice, but further studies are necessary to determine whether this increase of etonogestrel effect by 5-HT extends to mutant mice.

Disclosures: A.R. Casciato: None. **L. Bianchi:** None. **N. Ramanantsoa:** None. **B. Matrot:** None. **P. Cardot:** None. **J. Gallego:** None. **F. Cayetanot:** None. **L. Bodineau:** None.

Poster

231. Respiration: Modulation and Regulation

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 231.22/O42

Topic: E.08. Respiratory Regulation

Support: French National Agency for Research (ProgestVentil; ANR-15-CE17)
Chancellerie des Universités de Paris (Legs Poix 1504)
Association Française pour le syndrome d'Ondine

Title: Orexin neurons contribute to central modulation of the respiratory drive by progestins on *ex vivo* rodent preparations, a potential outcome for central hypoventilations

Authors: C. LOISEAU, A. R. CASCIATO, F. CAYETANOT, ***L. BODINEAU**;
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Abstract: Introduction Congenital central hypoventilation syndrome (CCHS) is a neurorespiratory disease characterized by a dysfunction of the CO_2/H^+ chemosensitivity that induces life-threatening hypoventilations. No pharmacological treatment is available. Clinical observations revealed a recovery of the CO_2/H^+ chemosensitivity in two adult CCHS female patients under desogestrel, a potent progestin used for contraceptive purpose. We hypothesized that the active metabolite of desogestrel (etonogestrel, ETO), is able to induce the recovery of CO_2/H^+ chemosensitivity by (over-)activating CO_2/H^+ sensitive central structures still functional. However, except the fact that supramedullary regions may be involved, the mechanisms are still unknown. **Materials and Methods** ETO effect on acidosis-induced increase in respiratory frequency (f_R) was appreciated on *ex vivo* preparations from newborn rats with different rostral extensions. Based on these results, the following experiments were made on diencephalic brainstem spinal cord (DBS) preparations: 1/ immunohistochemical detection of c-FOS in brainstem and diencephalic regions, 2/ comparison of the ETO effect under exposure to almorexant, a specific antagonist of orexin receptors, and 3/ c-FOS and orexin dual immunohistochemical detection on DBS preparations exposed or not to almorexant. **Results** We observed that the reinforcement of the respiratory response to metabolic acidosis by ETO occurred in a small range of concentration and required necessarily the presence of the diencephalon. c-FOS immunohistochemical detection revealed that several brainstem respiratory structures were over-activated and *de novo* activated in presence of ETO, such as the ventrolateral medullary reticular nucleus (VLM). We then demonstrated that the diencephalic orexinergic neurons constitute a key neuronal population in the ETO effect: ETO exposure significantly increased the number of c-FOS/orexin-containing neurons and blocking the orexinergic signalisation resulted in the loss of both the strengthening of the respiratory response to metabolic acidosis and the (over-)activation of brainstem respiratory structures. **Conclusion** Our results suggest that ETO reinforces the respiratory response to metabolic acidosis by (over-)activating brainstem structures, which exert collectively a facilitatory influence on the VLM and thus on the respiratory drive. To date the revealed respiratory-related structures do not seem to be deficient in CCHS patients, thus we assume that our results highlighted, at least in part, a neuronal pathway used by ETO to induce a recovery of the CO_2/H^+ chemosensitivity in patients.

Disclosures: C. Loiseau: None. **A.R. Casciato:** None. **F. Cayetanot:** None. **L. Bodineau:** None.

Poster

231. Respiration: Modulation and Regulation

Location: Hall A

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Program #/Poster #: 231.23/O43

Topic: E.08. Respiratory Regulation

Support: FAPESP
CAPES
NIH

Title: PHOX2B mutation mediated by Atoh-1 expression in the retrotrapezoid nucleus impaired respiratory rhythm and ventilatory responses to hypoxia

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Abstract: Retrotrapezoid nucleus (RTN) neurons modulate central chemoreception and respiratory automaticity. Phox2b and Atoh-1 expression characterizes these neurons. Germline Phox2b mutations result in congenital central hypoventilation syndrome, producing an impaired respiratory response to hypercapnia and hypoxia. Our goal was to investigate whether a conditional mutation of Phox2b driven during Atoh-1 expression in RTN neurons might affect a) respiratory rhythm; b) ventilatory responses to hypercapnia or hypoxia and c) number of RTN neurons. Here, we used a transgenic mouse line carrying a conditionally expressed, humanized PHOX2BΔ8 mutation. We crossed them with Atoh-1-cre mice. Thus, experimental group was selected based in the presence of both phenotypes: Atoh-1-cre^{+} and PHOX2BΔ8^{+} (n=8; male and female). Cre-negative mice were used as control (n=8; male and female). Ventilation recordings performed by whole body plethysmograph occurred in adult life (P30-40). Basal recordings were done in room air. To test respiratory chemoreflex responses, mice were submitted to hypercapnia (7%CO_2) or hypoxia (8%O_2) during 10 min each challenge. Anatomically, RTN neurons can be defined by Phox2b expression and absence of tyrosine hidroxilase (TH). These studies were followed by Phox2b^{+}/TH^{-} neuron immunohistochemical quantification. In room air, experimental and control groups showed similar respiratory parameters with exception to inspiratory (0.081 ± 0.003 s *vs*. control: 0.094±0.003 s; $p<0.01$) and expiratory time (0.21 s ± 0.006 s *vs*. control: 0.18± 0.004; $p<0.01$). Respiratory rhythm was analyzed by Poincare plot and showed an increase of breath irregularity after Phox2b mutation. In relation to chemoreflex, the peak change of respiratory responses to hypercapnia was similar between groups. In contrast, the increase in tidal volume and minute ventilation during hypoxia stimulus were significantly reduced in experimental group. Preliminary results showed that Phox2b mutation induced a

reduction of 20% of total Phox2b^{+}/TH^{-} neurons. Our data indicates that PHOX2BΔ8 mutation in Atoh-1 expressing cells compromised respiratory rhythm, respiratory response to hypoxia and RTN neuron number.

Disclosures: **C.B. Ferreira:** None. **P.E. Silva:** None. **F.C. Sousa:** None. **T.M. Silva:** None. **J.J. Otero:** None. **T.S. Moreira:** None. **A.C. Takakura:** None.

Poster

231. Respiration: Modulation and Regulation

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Program #/Poster #: 231.24/O44

Topic: E.08. Respiratory Regulation

Support: FAPESP 2016/23513-1; 2015/23467-7; 2013/17251-6
CNPq 425586/2016-2

Title: Maternal obesity promotes active expiration and increases baseline sympathetic activity in offspring

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Abstract: Maternal obesity is an epidemiological pathology that can promote several diseases in the offspring, including cardiovascular and respiratory dysfunctions, mainly due to changes sympathetic activity by neural mechanisms not yet elucidated. In the present study, we evaluated the changes in the respiratory pattern and their impact on the sympathetic outflow in offspring of dams treated with a high-fat diet (HFD - 45% calories from fat) during 6 weeks before gestation until lactation. After weaning, male offspring of standard diet dams (O-SD, n = 6-10) or HFD dams (O-HFD, n = 6-11) were fed with SD until the experimental day (P28-32). Using an arterially-perfused *in situ* preparation, O-SD and O-HFD rats were surgically prepared to record the thoracic sympathetic (tSN), phrenic (PN), hypoglossal (HN) and abdominal nerve (AbN) activities associated with extracellular recordings of RVLM presympathetic neurons under basal conditions and hypercapnia challenge (10% CO_2). In baseline conditions, O-HFD rats exhibited anticipated HN pre-inspiratory activity (pre-I; O-HFD: 0.63 ± 0.08, vs. O-SD: 0.39 ± 0.06 s, $p < 0.05$), associated with the presence of active expiration (late-E) (O-HFD: 7.2 ± 1.5, vs. O-SD: 1.3 ± 0.4 events/min, $p < 0.01$). O-HFD rats also presented augmented mean tSN activity (O-HFD: 43.7 ± 8.4, vs. O-SD: 21 ± 1.5 μV, $p < 0.05$) and blunted of respiratory-sympathetic coupling. During hypercapnia, the increase in HN pre-I activity was similar between O-SD and O-HFD, however, O-HFD presented an earlier onset of AbN late-E bursts relative to phrenic activity (O-SD: 0.57 ± 0.03, vs. O-HFD: 0.82 ± 0.06 s, $p < 0.05$) and increased mean tSN (O-SD: 120 ± 6.5,

vs. O-HFD: 147 ± 8 %, $p < 0.05$) We also observed that in the O-HFD, 3/6 neurons recorded in the RVLM have a late-E modulation in the baseline conditions and during hypercapnia, whereas none of the 6 neurons recorded in the RVLM of O-SD presented this modulation. These results suggest that maternal obesity critically affects fetal and post-natal development of respiratory-sympathetic network in offspring.

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Poster

231. Respiration: Modulation and Regulation

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Program #/Poster #: 231.25/P1

Topic: E.08. Respiratory Regulation

Support: HL104101
HL142227
HL137094

Title: Astrocyte Kir4.1 channels contribute to the ventilatory response to CO2/H+ in the retrotrapezoid nucleus

Authors: C. CLEARY[1], B. FLYNN[2], P. ROBSON[2], ***D. K. MULKEY**[1];
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Abstract: Central chemoreception is the mechanism by which the brain senses changes in tissue CO_2/H^+ to regulate breathing. A brainstem region called the retrotrapezoid nucleus (RTN) is an important site of chemoreception. Neurons in this region are intrinsically sensitive to CO_2/H^+; however, their activity is also subject to modulation by various transmitters including CO_2/H^+-evoked ATP release from local chemosensitive astrocytes through connexin hemichannels. In addition, astrocyte chemoreception appears to be a specialized feature of the RTN, since astrocytes at other levels of the respiratory circuit do not exhibit Ca+2 responses to CO2/H+. However, it is not clear whether all astrocytes within the RTN share this specialized function. Furthermore, pharmacological evidence suggests that RTN astrocytes sense CO2/H+ by inhibition of a Kir4.1-like conductance; however, the relevance of Kir4.1 channels to astrocyte chemoreception remain unclear. The goals of this project are to characterize astrocyte diversity within the RTN and to definitively test the role of Kir4.1 channels in astrocyte chemosensitivity and central chemoreception. By performing single cell RNA sequencing from acutely dissociated RTN cells from 9 day old C57BL/6J mouse pups, we identified two populations of mature astrocytes that share several common astrocyte markers, albeit at varying levels, (GFAP, Aldh1L1, Aqp4) but differ in expression of certain genes associated with astrocyte

chemoreception, including *gjb2* which encodes connexin 26, a CO2 gated connexin hemichannel. These results suggest that a discrete subset of RTN astrocytes function as chemoreceptors. To determine the relevance of astrocyte Kir4.1 channels to control of breathing, we generated an inducible astrocyte-specific Kir4.1 knockout (Kir4.1 cKO). Single cell RNA sequencing of RTN tissue from 10 day old Kir4.1 cKO and control pups show a nearly identical pattern of gene expression across all cell types except for the putative chemoreceptor cluster in which ~40 genes were upregulated including *gjb2*. Despite increased expression of *gjb2*, Kir4.1 cKO mice exhibit a pronounced respiratory deficit during exposure to graded increases in CO2. To determine whether loss of Kir4.1 from RTN astrocytes contribute to this respiratory phenotype, we used an AAV viral delivery system to over-express Kir4.1 channels in RTN astrocytes in Kir4.1 cKO mice. Preliminary results suggest that this approach improved CO2-dependent respiratory output in Kir4.1 cKO animals. These data identify Kir4.1 channels as requisite determinants of astrocyte chemoreception.

Disclosures: C. Cleary: None. **B. Flynn:** None. **P. Robson:** None. **D.K. Mulkey:** None.

Poster

231. Respiration: Modulation and Regulation

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Topic: E.08. Respiratory Regulation

Support: NIH Grant HL104101
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NIH Grant HL137094

Title: P2Y2 receptors contribute to the specialized CO2/H+ regulation of vascular tone in the retrotrapezoid nucleus

Authors: *C. CLEARY[1], T. S. MOREIRA[2], A. T. TAKAKURA[3], D. MULKEY[1]; [1]Univ. of Connecticut, Storrs, CT; [2]Univ. of Sao Paulo, Sao Paulo, Brazil; [3]Dept of Pharmacology, Inst. of Biomed. Science, Univ. of Sao Paulo, Sao Paulo, Brazil

Abstract: There is a close relationship between mechanisms regulating breathing and blood flow. For example, the same stimulus that drives breathing (e.g., CO_2/H^+, sensed by so called respiratory chemoreceptors) also acts as a vasodilator. However, since vasodilation may decrease tissue CO2/H+ and counter-regulate chemoreceptor activity, we wondered whether chemoreceptor regions have adapted a means of preventing CO2/H+-induced vasodilation. Consistent with this, we showed previously that CO2/H+-dependent regulation of vascular tone in a chemoreceptor region, the retrotrapezoid nucleus (RTN), is opposite to the rest of the cerebrovascular tree; exposure to CO2/H+ decreases RTN arteriole tone by a purinergic-

dependent mechanism. However, the cellular and molecular basis for specialized purinergic-dependent regulation of vascular tone in the RTN is unknown. The first goal of this study is to characterize cellular expression of all P2-purinergic receptors at the astrocyte-arteriole interface in chemoreceptor regions including the caudal nucleus tractus solitarius (cNTS), raphe obscurus (ROb) and RTN. Each region was isolated from endothelial and smooth muscle-specific mouse reporter lines for FACS sorting and bulk qPCR. Several P2X and P2Y receptors were detected in each region, however, only P2Y2 showed an RTN expression pattern across cell types that favored constriction. Specifically, P2Y2 transcript was expressed 6.8-fold higher than control in smooth muscle cells and 9.2-fold lower than control in endothelial cells. The second goal of this study is to assess the functional relevance of P2Y2 receptors using video microscopy and whole animal plethysmography. Exposure to a 10% increase in CO2 constricted arterioles in RTN brain slices by 11.2% but dilated arterioles in the NTS and ROb by 6.0% and 6.5%, respectively. The vascular response of RTN arterioles was eliminated (0.8% change) by incubation in a P2Y2 selective blocker AR-C118925 and mimicked by a selective P2Y2 agonist PBS1114 (10.5% constriction). Similarly, we found in urethane-anesthetized mice that exposure to high CO2 decreased RTN pial vessel tone and this response was blocked by application of AR-C118925 and mimicked by application of PBS1114. Furthermore, preliminary results also suggest condition smooth muscle P2Y2 knockout mice have a significantly blunted ventilatory response to CO2. These results identify P2Y2 receptors in RTN vascular smooth muscle cells as requisite determinants of CO2/H+ vascular reactivity and the ventilatory response to CO2.

Disclosures: **C. Cleary:** None. **T.S. Moreira:** None. **A.T. Takakura:** None. **D. Mulkey:** None.

Poster

231. Respiration: Modulation and Regulation

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Program #/Poster #: 231.27/P3

Topic: E.08. Respiratory Regulation

Support: HL122921
T32NS041234

Title: Leptin receptor-expressing neurons in the NTS match breathing to diet-induced increases in body mass

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Abstract: A remarkable characteristic of the respiratory system is the matching between ventilation and metabolism which is so precise that there is almost no change in arterial blood

gases in the presence of moderate variations in metabolism. Hence, chemoreceptor activation cannot explain the ventilatory response. We speculate the existence of a neural circuit that receives chemosensory information but also receives information related to metabolism. The caudal nucleus of the solitary tract (NTS) receives dense innervation from sensory afferents of the vagal and glossopharyngeal nerve and there is a group of cells in the NTS that express the functional long-form leptin receptor (LepRb). Leptin is known to stimulate breathing, and as leptin also increases energy expenditure, the concomitant change in respiration is essential to maintain arterial blood gas homeostasis. We have previously shown that leptin receptors are expressed on neurons in the NTS and that the electrophysiological properties identify two neuron types, types 1 and 2. Type1 cells co-express galanin and leptin depolarizes these cells by activating a non-selective cationic current carried by NALCN. In continuation, we have characterized the projection patterns of LepRb-expressing NTS neurons. Also, we conducted plethysmographic measurements of breathing in freely-moving awake mice in which NALCN is selectively deleted in LepRb-expressing cells. These mice exhibited irregular breathing patterns and upon being fed for up to 3 weeks on a high fat diet, exhibited a depressed minute ventilation. Lastly, we are using a chemogenetic approach to test whether selectively inhibiting LepRb-expressing NTS neurons depresses breathing. Together, our data suggest that LepRbexpressing NTS neurons form a circuitry that integrates leptin-mediated metabolic state with the control of breathing.

Disclosures: J. Do: None. **M. Martina:** None. **D.R. McCrimmon:** None.

Poster

232. Vocalization and Social Behavior in Songbirds I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 232.01/P4

Topic: F.01. Neuroethology

Support: NSF Grant IOS1456965

Title: A software tool for determining subthreshold ionic currents in HVC neurons of the zebra finch

Authors: A. DAVENPORT[1], R. L. HYSON[2], F. JOHNSON[2], *R. BERTRAM[1];
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Abstract: The resting membrane potential of a neuron is determined primarily by subthreshold currents, including the leakage current (I_L) and the hyperpolarization-activated current (I_h). These currents also dictate the response of the neuron to hyperpolarizing stimuli. In neurons of the male zebra finch HVC, the brain nucleus that encodes the bird's song, the h-current varies during development in response to song learning, and this intrinsic plasticity likely plays a role in the

learning process. We have developed biophysical models to aid in the study of learning-related changes in I_h. The model contains several parameters related to I_h and I_L conductance, including their maximal conductance and the activation properties of I_h. Here, we describe a software tool that determines these parameter values in an automated fashion, using current-clamp data from the target neuron. This tool performs a fast optimization that minimizes the difference between the model voltage trace and that of the current-clamped neuron under various conditions. Because of the rapidity of the calculation, it can be performed while the neuron remains patched, providing predictions that can be utilized on the same neuron with tools such as Dynamic Clamp. Though developed for HVC neurons, the software can be applied to any neuron type to quantify I_h and I_L currents using whole-cell current-clamp data.

Disclosures: A. Davenport: None. **R.L. Hyson:** None. **F. Johnson:** None. **R. Bertram:** None.

Poster

232. Vocalization and Social Behavior in Songbirds I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 232.02/P5

Topic: F.01. Neuroethology

Support: Department of Science and Technology (DST), India to SI (grant # SR/SO/AS-39/2009)
NBRC Core funds

Title: Role of mu-opioid receptors in the motivation to sing and acoustic features of female-directed songs in male zebra finches

Authors: *S. KUMAR[1], A. N. MOHAPATRA[1], U. DIN[1], H. SHARMA[2], S. SHARMA[1], U. A. SINGH, Jr[1], V. ARORA[1], N. KAMBI[1], A. DUTTA[1], T. VELPANDIAN[2], R. RAJAN[3], S. IYENGAR[1];
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Abstract: Objective and rationale: Mu-opioid receptors (μ-ORs) are G-protein-coupled receptors found throughout the nervous system and bind endogenous opioid peptides such as enkephalin and endorphin. High levels of μ-ORs are expressed in different components of the basal ganglia, which receives dopaminergic projections from the VTA/SNc. Dopamine is known to affect the inhibitory output of the basal ganglia and modulate the cortico-basal ganglia-thalamic circuitry. This circuitry is involved in various cognitive functions such as motivational and motor aspects of behaviour, including vocalization across different species of vertebrates. Interestingly, modulating levels of the endogenous opioids or their binding in the basal ganglia can change dopamine release by the VTA/SNc and affect associated behaviours. Songbirds like zebra

finches show high expression of μ-ORs in various song control regions. Social context-dependent singing is a direct output of a part of this circuitry (called anterior forebrain pathway or AFP), making songbirds an excellent model system to study the role of opioid modulation in vocal behaviours.
Methods: To study the role of opioid neuromodulation on female-directed (FD) singing in zebra finches which is a male courtship behaviour, we performed site-specific infusions of μ-OR antagonists in the nuclei of the AFP by microdialysis and analysized the neurotrasmitters by HPLC-MS in the dialysate, while simultaneously recording the behaviour of experimental birds. This methodology gave us direct insights (from the molecular to the behavioural level) as to how the endogenous opioid system directs motivational aspects of singing during courtship in zebra finches.
Results: Inhibiting μ-ORs in Area X (a basal ganglia homologue in songbirds of AFP) using antagonists results in a significant dose-dependent increase in the number of FD songs which suggests an increase in the motivation to sing. Interestingly, the same manipulation in the cortical nucleus LMAN which is upstream of Area X led to a decrease in the number of songs. Further, we found changes in the acoustic features of individual syllables that these birds sang, after the μ-OR system was modulated in Area X.
Conclusions: Our results confirm that μ-ORs in the AFP play a major role in the motivation of male birds to sing FD songs. Also, we report for the first time that μ-OR modulation in different components of the AFP can affect the acoustic properties of songs.

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Poster

232. Vocalization and Social Behavior in Songbirds I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 232.03/P6

Topic: F.01. Neuroethology

Support: NSF Grant IOS 1656360

Title: Experience-dependent changes in I_h in neurons that contribute to learned vocalizations

Authors: *A. BRUNICK[1], R. BERTRAM[2], F. JOHNSON[1], R. L. HYSON[1];
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Abstract: Juvenile male zebra finches learn to produce structured vocalizations during and after forming an auditory memory of an adult tutor's song. HVC is a cortical premotor nucleus necessary for these processes. Some HVC neurons project to Area X, a basal ganglia structure,

that indirectly modulates the vocal-motor nucleus RA. HVC_X projection neurons have electrophysiological features that are distinct from other HVC neurons. One such feature of adult HVC_X neurons is a voltage sag appearing during prolonged hyperpolarizing current pulses. This feature is the result of the h-current (I_h), which is mediated by hyperpolarization activated, cyclic nucleotide gated channels (HCN). Juvenile birds show an experience-dependent suppression of sag. Biophysical models of these recordings suggested that tutor exposure transiently suppresses I_h. To test this prediction, whole cell recordings were taken from HVC_X neurons of tutored and tutor deprived juveniles. I_h was blocked with bath application of ZD7288 and dynamic clamp was used to electrically reintroduce modeled I_h in an attempt to rescue the pre-ZD7288 response. ZD7288 blocked the voltage sag, but also had effects such as increased voltage drop, decreased rebound depolarization, and a more hyperpolarized resting potential. Effects of ZD7288 were seen in all cells, regardless of the presence of sag, demonstrating that I_h is present in HVC_X neurons of juveniles raised with and without a tutor, and that tutor exposure may not only affect the overall conductance of I_h, but also I_h kinetics. I_h has both fast and slow components, the balance of which depends on HCN subunit composition. Hence, it is possible that tutor exposure alters the subunit composition of HCN channels. Addition of modeled I_h via dynamic clamp rescued several features affected by ZD7288: sag, rebound depolarization, and voltage drop, providing some validation of our I_h models. Resting potential, however, was not restored to original values. This suggests that the contribution of I_h to resting potential is not fully accounted for by our original models or that ZD7288 has non-specific effects on other currents involved in maintaining the resting potential.

Disclosures: **A. Brunick:** None. **R. Bertram:** None. **F. Johnson:** None. **R.L. Hyson:** None.

Poster

232. Vocalization and Social Behavior in Songbirds I

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Topic: F.01. Neuroethology

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National Institutes of Health Grant R01 EB022872

Title: Neural circuit mechanisms underlying different forms of vocal learning in adult songbirds

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Abstract: Complex behaviors are learned and optimized through multiple mechanisms. One such mechanism is the process of sensorimotor error correction, wherein the brain uses sensory feedback to adjust motor output to improve behavioral performance. Another mechanism is the reinforcement of a behavior via rewarding or aversive feedback. It is unclear whether the brain engages similar or disparate neural circuits for different forms of motor learning or when feedback cues are provided by different sensory modalities. Adult Bengalese finches (Lonchura striata var. domestica) perform a learned, skilled behavior (song), acquired through a sensorimotor learning process that requires a canonical thalamocortical-basal ganglia neural circuit. Prior research has focused on the importance of sensorimotor error correction for song learning, specifically by using auditory cues to drive changes in vocal output. We developed a novel learning paradigm in songbirds by delivering a non-auditory, pitch-contingent, aversive cue (electric stimulation) during singing. This stimulus reliably drives significant adaptive shifts in pitch of the targeted song syllable. We will probe the necessity of a songbird thalamocortical-basal ganglia circuit for both aversive cue-driven vocal learning and sensorimotor error correction. These results will uncover the neural circuitry important for different forms of motor learning.

Disclosures: J.N. McGregor: None. **P.I. Jaffe:** None. **A.L. Grassler:** None. **A.L. Jacob:** None. **M.S. Brainard:** None. **S.J. Sober:** None.

Poster

232. Vocalization and Social Behavior in Songbirds I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 232.05/P8

Topic: F.01. Neuroethology

Support: NIH R01 NS099375
NIH R01 NS084844
NSF Grant 1822677
MCKNIGHT FOUNDATATION TECH INNOVATIONS IN NEUROSCIENCE

Title: Neuromotor agility in the songbird: Tools and goals

Authors: *A. PACK[1], J. S. YAN[2], A. N. WOOD[1], K. H. SRIVASTAVA[1], M. PASQUALI[2], C. P. ELEMANS[3], S. J. SOBER[1];
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Abstract: A central goal of neuroscience is to discover how neurons control muscles to produce complex behaviors. In pursuit of this goal, we developed novel hardware and algorithmic approaches to investigate motor systems function in songbirds. We developed two novel

methods to record electromyographic signals to understand how spiking patterns in motor units, where one motor unit consists of one motor neuron and the muscle fibers it innervates, shape behavior. The first method uses a multi-channel microelectrode flexible array sutured to the surface of targeted vocal muscles. The second method uses carbon nanotube fibers inserted under the fascia surrounding the muscles. Both electrode systems allow us to chronically record single- and multi-unit activity from small (2-4 mm long) vocal muscles without damaging the vocal organ or interfering with normal vocal behavior. Combining these electrode tools with recordings of neural activity in vocal motor cortex during song and with novel algorithms for identifying the precisely-timed spike patterns that underlie skilled motor behavior, we demonstrated that both neurons in cortex and motor units in vocal muscles use millisecond-precise, multi-spike timing patterns to control behavior. Moreover, we found that experimentally-induced variation in spike timing patterns significantly alter muscle force output in both breathing and vocal behavior. Ongoing and future studies investigate how motor codes in muscles change during learning to evaluate the hypothesis that vocal skill learning relies on the nervous system's ability to organize precisely timed patterns of spiking activity.

Disclosures: **A. Pack:** None. **J.S. Yan:** None. **A.N. Wood:** None. **K.H. Srivastava:** None. **M. Pasquali:** None. **C.P. Elemans:** None. **S.J. Sober:** None.

Poster

232. Vocalization and Social Behavior in Songbirds I

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Topic: F.01. Neuroethology

Support: National Institutes of Health Grant R01 NS099375
National Institutes of Health Grant R01 NS084844
MCKNIGHT FOUNDATATION TECH INNOVATIONS IN NEUROSCIENCE

Title: Neuromotor agility in the songbird: Flexible multielectrode arrays with 3-dimensional contacts to enhance electromyogram recordings

Authors: *M. ZIA[1], B. CHUNG[2], M. BAKIR[1], S. J. SOBER[3];
[1]Georgia Inst. of Technol., Atlanta, GA; [3]Biol., [2]Emory Univ., Atlanta, GA

Abstract: A central goal of neuroscience is to discover how neural circuits drive complex activation patterns in muscles to control complex behaviors. However, despite recent advances in tools for monitoring and manipulating neural activity, methods for recording and analyzing the physiological signals that actually control behavior - spiking activity in muscle fibers - have changed little in recent decades. Consequently, our understanding of how the brain controls the body remains limited. To address these challenges, we developed a platform for fabricating

flexible multi-electrode arrays (MEAs) with 3-dimensional (3D) contact sites for recording single-unit activity from the muscle surface. A unique fabrication process was utilized for 3D metal deposition with gold coating on flexible biocompatible materials (polyimide and PDMS). The flexibility enabled the arrays to move with the muscles they were implanted on. The 3D contact sites of the MEA enhanced the signal-to-noise ratio of the devices, as did careful selection of the size and spacing of individual electrode contact sites. The unique fabrication process allowed for scalability of the electrode sites in density, count, and height and enabled rapid prototyping for a host of applications across different species and motor effectors.

Disclosures: M. Zia: None. **B. Chung:** None. **M. Bakir:** None. **S.J. Sober:** None.

Poster

232. Vocalization and Social Behavior in Songbirds I

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Program #/Poster #: 232.07/P10

Topic: F.01. Neuroethology

Support: NIH Grant R01-NS099375
NIH Grant R01-NS084844
McKnight Foundation Tech Innovations in Neuroscience

Title: Neuromotor agility in the songbird: Distributed spike codes across populations of motor units

Authors: *B. CHUNG[1], R. E. CONN[1], M. ZIA[4], M. BAKIR[5], I. NEMENMAN[2], S. J. SOBER[3]; [1]Biol. Dept., [2]Physics Dept., [3]Biol., Emory Univ., Atlanta, GA; [5]Electrical and Computer Engin., [4]Georgia Inst. of Technol., Atlanta, GA

Abstract: Skilled motor behaviors are controlled by large populations of neurons driving a smaller number of muscles. Neural activity must therefore be coordinated across converging populations of units in order to control motor outputs. While spike rates during behavior have been shown to be correlated across neurons, in vertebrates the effect of one neuron's activity on behavior is very small. New work from our lab, however, shows that precisely-timed millisecond-scale spike patterns are sufficient to drive behavior in songbirds, raising the question of whether and how much precise spike timing is coordinated across neurons and motor units. Here we use information-theoretic approaches to show that similar to spike rates, precise spike timing patterns are correlated across simultaneously-recorded motor units, and that these multi-unit patterns predict the dynamics of behavior. Further analysis will examine the diversity of motor codes across motor units with different firing properties.

Disclosures: **B. Chung:** None. **R.E. Conn:** None. **M. Zia:** None. **M. Bakir:** None. **I. Nemenman:** None. **S.J. Sober:** None.

Poster

232. Vocalization and Social Behavior in Songbirds I

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Support: National Institutes of Health Grant R01 NS099375
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MCKNIGHT FOUNDATATION TECH INNOVATIONS IN NEUROSCIENCE
NSF GRFP DGE-1444932

Title: Neuromotor agility in the songbird: Efficient algorithms for data analysis

Authors: *R. E. CONN[1], B. CHUNG[1], N. J. MARSHALL[4], M. M. CHURCHLAND[4], I. NEMENMAN[2,3], S. J. SOBER[3];
[2]Physics, [3]Biol., [1]Emory Univ., Atlanta, GA; [4]Neurosci., Columbia Univ., New York, NY

Abstract: Neurons and motor units (muscle fibers innervated by a single motor neuron) must coordinate their activity to produce behavior. Neurons and motor units transmit information about behavior by modulating their spike patterns across a range of fast (milliseconds) and slow timescales. Recent work from a number of groups has demonstrated the importance of milliseconds-scale spike timing in motor control, particularly in the muscles that control skilled behaviors. However, three persistent challenges in decoding motor activity at fast timescales are: 1) to identify and sort spike waveforms recorded from multiple motor units, 2) to effectively estimate predictive relationships between continuous variables, such as precise spike times and behavioral measures, and 3) to determine the error sensitivity of such measures. Overcoming the challenges of accuracy, optimization, and robustness, we developed an analysis pipeline for implementing the steps outlined above. Individual spikes were sorted using a novel algorithm that employs Bayesian non-parametrics and optimal filtering to identify individual units from recorded data. In order to determine whether the activity of single motor units at fast and slow timescales is predictive of behavior, information-theoretic approaches have been employed using a k-nearest neighbors estimator of mutual information for continuous variables (Kraskov, Phys Rev E, 2004). In order to extend this analysis to multiple neurons we need to overcome inconsistencies of dimensionality by partitioning data. Consequently the number of iterations necessary for accurate estimation grows significantly. We develop a toolbox to run iterations and optimize parameters of the estimator in parallel across multiple CPUs, which results in much shorter compute times when compared to serial processing. Finally, we generate surrogate

datasets -- including synthetic spike trains for which ground truth is known -- to send through the pipeline, allowing us to assess the robustness of each stage to errors in the detection of spikes.

Disclosures: R.E. Conn: None. **B. Chung:** None. **N.J. Marshall:** None. **M.M. Churchland:** None. **I. Nemenman:** None. **S.J. Sober:** None.

Poster

232. Vocalization and Social Behavior in Songbirds I

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Program #/Poster #: 232.09/P12

Topic: F.01. Neuroethology

Support: National Science Foundation DGE-1444932
National Institutes of Health Grant R01 NS099375
National Institutes of Health Grant R01 NS084844
National Institutes of Health Grant R01 EB022872

Title: Effects of dopamine depletion on neural activity in the songbird basal ganglia

Authors: *A. N. WOOD, A. L. JACOB, S. J. SOBER;
Biol., Emory Univ., Atlanta, GA

Abstract: Dopaminergic signaling in the basal ganglia (BG) has long been implicated as an important mechanism for motor learning across a wide range of species, and loss of dopaminergic cells in humans leads to Parkinson's disease, a disorder characterized by motor impairments. Songbirds provide an excellent model system for studying the effects of dopamine loss in the BG during motor performance due to the presence of a well-defined and anatomically discrete BG nucleus, Area X, which is exclusively involved in singing behavior. Our lab has previously shown that lesions of the dopaminergic inputs to Area X in the Bengalese finch (*Lonchura striata*) leads to a impairment in song learning (Hoffmann et al. 2016, *Journal of Neuroscience*) and subtle changes in song production. However, it is currently unknown how loss of dopamine affects the neurophysiology of Area X neurons. We will combine single-unit neurophysiology in Area X with 6-hydroxydopamine lesions to examine the role of dopaminergic depletion on neural activity during vocal behavior.

Disclosures: A.N. Wood: None. **A.L. Jacob:** None. **S.J. Sober:** None.

Poster

232. Vocalization and Social Behavior in Songbirds I

Location: Hall A

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Program #/Poster #: 232.10/P13

Topic: F.01. Neuroethology

Support: NIH 1R01MH117778
NIH R01DC13826
NIH R01NS099288
NSF 1354962

Title: Inferring latent descriptions of animal vocalizations

Authors: *J. GOFFINET, S. BRUDNER, V. MICHAEL, J. SINGH ALVARADO, T. HARMON, K. TSCHIDA, R. MOONEY, J. PEARSON;
Duke Univ., Durham, NC

Abstract: Vocalization is a complex behavior that underlies vocal communication and vocal learning, and is important for the study of humans' underlying linguistic competency and musicality. Yet despite wide interest from a number of disciplines, quantitative approaches to the comparative study of animal vocalization have been stymied by a reliance on small sets of handpicked features used to describe complex acoustical data. These features are most often determined within species-specific research communities for the purpose of discriminating among subpopulations and experimental conditions. Consequently, they both imperfectly capture the distribution of natural vocalizations and prohibit comparisons across species, genera, and larger clades. However, recent advances in machine learning have resulted in techniques that allow high-dimensional data to be compressed in a data-dependent manner, resulting in low-dimensional encodings that minimize information loss. Here, we use one such method, the variational Bayesian autoencoder (VAE), to perform dimension reduction of the vocalizations and vocal learning behavior of two model organisms: the laboratory mouse and the zebra finch, a small Australian songbird. We show that the latent representations of these species' vocal behavior can be used to answer questions about distributions of vocalizations with accurate uncertainty estimates. We apply these techniques to two common experimental assays--vocal learning and vocal performance--and show that in each case, latent representations allow us to accurately characterize vocal variability. Thus, for instance, we can directly compare, in a data-driven manner, distributions of vocalizations produced across experimental conditions and across groups, trial-to-trial variability in vocal production, and vocal adjustments during learning. As a result, such methods present new opportunities for the quantitative study of vocal behavior in a species-agnostic manner, offering a unified view of vocal variability and learning on timescales ranging from individual syllables of millisecond duration to days.

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Poster

232. Vocalization and Social Behavior in Songbirds I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 232.11/DP12/P14

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: F.01. Neuroethology

Support: F31MH110209

Title: Pharmacological or genetic reduction of miR-128 enhances learned vocal communication

Authors: *C. M. AAMODT[1], S. A. WHITE[2];
[1]Neurosci. Interdepartmental PhD Program, [2]Integrative Biol. and Physiol., UCLA, Los Angeles, CA

Abstract: Novel approaches for autism drug discovery are urgently needed to treat communication deficits in patients who are severely affected. Autism risk genes are enriched in miR-128 targets, and this microRNA is also aberrantly upregulated in postmortem tissue from autism patients. Given its relevance to the disorder, miR-128 may be a viable target for therapeutic development.
The zebra finch songbird is a widely used model for vocal learning and communication. Previously our lab generated an activity-dependent gene regulation network in the adult songbird striatopallidal song nucleus Area X to identify master regulators of singing behavior. Using this dataset we discovered that two of the genes most highly correlated to singing are the host genes for miR-128. *In vitro* studies have shown that a bioactive glycoside found in the cognitive enhancer ginseng, ginsenoside Rh2 (GRh2), modulates miR-128 levels. Using a microRNA qPCR assay we found that an acute oral dose of GRh2 robustly decreases miR-128 in Area X. We hypothesized that GRh2 would rescue communication deficits by decreasing miR-128.
To test this hypothesis, we first isolated songbirds during the critical period for vocal learning to generate adults with impaired song. Well after the normal critical period closure, isolated birds were returned to their parental home cage and treated daily with oral GRh2 (10mg/kg) or vehicle for four weeks. Birds that received GRh2 organized their syllables into stable sequences, whereas vehicle failed to enhance syllable sequencing.
We next used a siRNA sponge designed to decrease miR-128 levels in Area X during the critical period for song learning. Bilateral injection of the targeting siRNA construct into Area X was sufficient to enhance learned vocal sequencing in young songbirds relative to scramble controls. During the final phase of this project we will knock down miR-128 in Area X of adult social

isolates to determine whether decreased miR-128 is sufficient to recapitulate the therapeutic effects of GRh2 on birds with vocal communication deficits. These results suggest that the molecular mechanisms underlying speech and language can be pharmacologically and genetically targeted to accelerate the development of novel therapeutics for disorders like autism and intellectual disability.

Disclosures: C.M. Aamodt: None. **S.A. White:** None.

Poster

232. Vocalization and Social Behavior in Songbirds I

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Program #/Poster #: 232.12/P15

Topic: F.01. Neuroethology

Support: NIH Grant R01NS104925
NIH Grant R01NS089679
Nvidia investigator grant

Title: Calcium imaging and machine learning tools for birdsong annotation reveal stability and neural correlates of canary song syntax

Authors: *Y. COHEN[1], J. SHEN[1], D. SEMU[1], D. P. LEMAN[1], W. A. LIBERTI, III[2], N. PERKINS[1], D. A. NICHOLSON[3], T. J. GARDNER[1];
[1]Biol., Boston Univ., Boston, MA; [2]Electrical Engin. and Computer Sci., UC Berkeley, Berkeley, CA; [3]Emory Univ., Atlanta, GA

Abstract: Songbirds are an excellent model for investigating the neural basis of learned sequential behaviors like tool use and communication. Their songs consist of a series of distinct vocalizations called syllables. Species such as Bengalese finches and canaries sing hundreds of times each day in the lab and produce flexible syllable sequences. Statistical models of these songs reveal that, like speech, certain element-to-element transitions are not first order Markov and, in canaries, reflect a memory of syllables sung tens of elements earlier in the sequence (Jin 2011, Markowitz 2013).
The sequence of song is largely governed by stereotyped syllable-locked activity in bursts of spikes in projection neurons in the premotor nucleus HVC (Hahnloser 2002, Long 2008, Wang 2008). To reconcile this neural precision with flexible sequence generation, models of HVC dynamics hypothesized a many-to-one mapping of HVC states onto syllables (Jin 2009). In these models a syllable can be driven by several “hidden” states and the dynamics is first order Markov - depending only on the current state. Evidence for hidden states was found in Bengalese finches where both HVC projection neurons and their premotor targets reflected selectivity to specific transitions (Fujimoto 2011, Wohlgemuth 2010).

We used head-mounted fluorescence microscopes to record song and Ca^{2+} signals from freely behaving canaries. Relating context-sensitive neural activity to properties of a rich syntax required data collection over months - yielding more than 5000 songs. Inspired by machine learning methods for human speech recognition, we developed a song segmentation and annotation algorithm, TweetyNet, that automated working with large datasets. Analyzing qualitatively-larger datasets of annotated song than previously possible, we confirmed previous reports of long-range order in canary song. Examining the activity of HVC projection neurons revealed hidden states that reflect key properties of canary syntax; Individual cells correlate with past and future contexts several seconds apart, show selectivity to more than a single context and their complex firing states aggregate in complex parts of the behavior, where song sequences are governed by memory-dependent transitions.

Disclosures: **Y. Cohen:** None. **J. Shen:** None. **D. Semu:** None. **D.P. Leman:** None. **W.A. Liberti:** None. **N. Perkins:** None. **D.A. Nicholson:** None. **T.J. Gardner:** A. Employment/Salary (full or part-time):; Neuralink.

Poster

232. Vocalization and Social Behavior in Songbirds I

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Topic: F.01. Neuroethology

Support: MEXT/JSPS grant 17H06380 (#4903), 17H01015

Title: *In vivo* calcium imaging of singing-related neural activities in premotor nuclei of Bengalese finches

Authors: *R. O. TACHIBANA, K. OKANOYA;
The Univ. of Tokyo, Tokyo, Japan

Abstract: Birdsong is a good model to study neural mechanisms for skilled motor sequences. Bengalese finches' songs consist of a sequence of various sound elements (syllables). Their songs have been reported to show stochastic syllable transitions at branching points in the sequence, as results of sequence analyses based on acoustical similarity among syllables. How does this syllable transition rule, or song syntax, reflect the sequential structure of internal premotor activities? The song motor pathway in the bird brain includes two premotor nuclei: HVC (high vocal center) and RA (robust nucleus of arcopallium). HVC neurons projecting to RA (HVC_{RA}) are known to exhibit burst firings sparsely at specific timings in song sequence, as a driving source of syllable productions. Thus, measuring activities of HVC_{RA} neurons during singing will reveal neuronal mechanisms for producing the motor sequence with such complex transition syntax. In the present study, we measured neuronal activities from multiple HVC_{RA}

neurons of freely-moving birds by the calcium imaging technique. Fluorescent calcium indicators (GCaMP) were expressed specifically in HVC_{RA} neurons using the adeno-associated viral vectors. After several weeks of waiting for the expression, we started to image spontaneous song productions by a light-weight miniaturized fluorescent microscope which was mounted onto bird's head. Preliminary results showed that each HVC_{RA} neuron fired at different timings in the song with reflecting the sequential transition pattern. Further analyses on consistency in transition patterns between the neuronal burst sequences and produced songs will be discussed.

Disclosures: R.O. Tachibana: None. **K. Okanoya:** None.

Poster

232. Vocalization and Social Behavior in Songbirds I

Location: Hall A

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Topic: F.01. Neuroethology

Support: NIH Grant GM120464

Title: Exploring portals in the zebra finch expression brain atlas (ZEBrA;www.zebrafinchatlas.org)

Authors: *P. V. LOVELL, C. V. MELLO;
Dept. of Behavioral Neurosci., Oregon Hlth. and Sci. Univ. Sch. of Med., Portland, OR

Abstract: Zebra finches (*T. guttata*), are a prime model organism for studying the biology of vocal learning. The Zebra finch Expression Brain Atlas (ZEBrA; www.zebrafinchatlas.org) is a public online resource for investigating the brain distribution of genes of relevance to the development and physiology of functional circuits in this species. This expanding collection presents high-resolution (0.46 µm/pixel) *in situ* hybridization digital images for ~660 genes expressed in the finch brain, along with annotated drawings from a reference atlas - a set of annotated drawings in registration with Nissl- and Myelin-stained sagittal sections. ZEBrA is organized into six thematic portals that facilitate examining genes of interest to songbird biology (e.g. song nuclei markers), speech and language disorders (e.g. FOXP2 and targets), human neurological diseases (OMIM-based), rodent behavioral and neurological phenotypes (MGI-based), comparative neuroanatomy (e.g. markers from the Allen's mouse brain atlas). Portal cross-referencing tools facilitate linking expression patterns in finches to behavioral phenotypes, neurological and psychiatric disorders, expression in non-avian species, and gene function. Recent ZEBrA-based explorations include: (1) Molecular architecture of the arcopallium: By analyzing arcopallial-expressed transcripts we have defined 20 distinct regions grouped into 6 major domains (Mello et al., JCN 2019). The data clarify the molecular organization of this key avian brain area, issues related to nucleus taenia, and similarities and differences between

cortical and amygdalar regions in birds and mammals. (2) Speech and Language: We have found that a subset of the 55 genes that are shared specializations of cortical vocal motor areas between vocal learning birds and humans(Pfenning et al., 2014) may be markers of general motor areas, thus clarifying the core set of specializations that are truly unique to the vocal circuitry. (3) Insights into Human Diseases: We have expanded the set of genes in ZEBrA related to speech and communication disorders (vocal dispraxias, stuttering), as well as neurological disorders (e.g. epilepsy, ataxias) helping to shed light into possible brain areas involved in these disorders. (4) Understanding the Avian Brain:Analyses of the expression patterns of mammalian cell type and regional-specific markers in the finch brain are revealing insights into the shared molecular signatures of specific brain areas between birds and mammals. (5) TRUST Certification: Team ZEBrA participated in an NIH workshop with a long-term goal of certifying ZEBrA as a trustworthy data repository.

Disclosures: P.V. Lovell: None. **C.V. Mello:** None.

Poster

232. Vocalization and Social Behavior in Songbirds I

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Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 232.15/P18

Topic: F.01. Neuroethology

Support: NSF 1557499
NIH NRSA T32

Title: Computing courtship: Song circuit modulation of mating posture in a female songbird

Authors: *A. PERKES, J. BURKE, N. KOLOTOUROS, K. DANIILIDIS, M. F. SCHMIDT; Univ. of Pennsylvania, Philadelphia, PA

Abstract: The song circuit is a set of well-defined brain nuclei that drive song production and learning in songbirds, however various findings suggest this circuit may also play a role in non-singing behavior. Female songbirds produce a copulation solicitation display (CSD) in response to male song. In brown-headed cowbirds (*Molothrus ater*), this copulatory posture can be evoked in isolation using song playback. Normal females produce more postures to songs of high potency, but prior experiments have shown that lesions targeted to the forebrain song circuit nucleus HVC disrupt this selectivity, resulting in females which produce postures to all conspecific songs. As CSD is not unique to songbirds, it is unlikely that the components of the song circuit directly drive CSD production. This suggests that the song system inhibits lower postural regions from generating CSD in response to low potency songs, and that disruptions to other regions within the song system would also disrupt selectivity without eliminating posture. Additional experiments are required to confirm this, and to assess how HVC encodes female

selectivity, as well as confirm that HVC–and not adjacent areas–modulates this response. In order to investigate the role of the song circuit in non-vocal copulatory displays, we produced a highly precise quantification of bird posture. From this, we observe that there exists subtle variation within CSD as a function of song potency. We developed a new, high-throughput experimental paradigm of assaying female preference, by playing songs to multiple birds simultaneously while monitoring motion to ensure a proper behavioral state. We use songs of known potency, which are ranked consistently by females, based on prior years of playbacks. To confirm that song selectivity is mediated by HVC output, we perform lesions in the song region RA (robust nucleus of the arcopallium), the downstream target of HVC and the primary output of the song circuit. Finally, to evaluate auditory responsiveness and selectivity in the song system, we record neural activity in HVC during song playback to head-fixed females. We test specifically whether auditory responses in HVC to song correlate with known behavioral potency.

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Poster

232. Vocalization and Social Behavior in Songbirds I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 232.16/P19

Topic: F.01. Neuroethology

Support: NSF Grant 143602

Title: Update on the NSF EDGE consortium project (2018-2019): “Developing gene manipulation tools and resources for a vocal learning species”

Authors: *C. V. MELLO[1], T. VELHO[2], A. KEYTE[3], A. Y. M. SOARES[2], M. T. BIEGLER[4], P. V. LOVELL[1], K. M. JUNG[5], J. Y. HAN[5], C. LOIS[6], E. D. JARVIS[3];
[1]Dept. of Behavioral Neurosci., Oregon Hlth. and Sci. Univ. Sch. of Med., Portland, OR; [2]Federal Univ. of Rio Grande do North, Natal, Brazil; [3]The Rockefeller Univ., New York, NY; [4]Neurobio., Duke Univ., Durham, NC; [5]Seoul Natl. Univ., Seoul, Korea, Republic of; [6]Div. of Biol. and Biol. Engin., Caltech, Pasadena, CA

Abstract: Studies in zebra finches, a songbird species, have made seminal contributions to our understanding of the behavioral and neural basis of vocal learning, a trait that subserves speech acquisition in humans. However, an in-depth understanding of the molecular genetics of vocal learning requires efficient gene manipulation tools, including the ability to make transgenic songbirds. Here we provide an update on the activities carried out by our collaborative NSF EDGE consortium to develop these tools. Activities and progress along 3 main lines are as

follows: 1) We are generating transgenic zebra finches that ubiquitously express the genome-editing enzyme Cas9, using our proven method of injecting VSVg-pseudotyped lentiviruses into freshly-laid fertilized eggs. These vectors contain Cas9 constructs under a strong ubiquitous promoter in CRE-dependent and independent forms designed to express Cas9 in all tissues. These lines are expected to have broad applicability, including brain gene manipulations through local injections of viral vectors with guide RNAs (gRNAs).Preliminary screening of mosaics indicates evidence of germline transmission of transgenes in F1 birds. 2) We are developing efficient protocols to isolate, culture, and utilize primordial germ cells (PGCs) for generating transgenic zebra finches. The ability to isolate and culture PGCs that become germ cells upon reintroduction into finch embryos will likely increase the efficiency and speed of generating finch transgenic lines. Maintaining PGCs *in vitro* would also facilitate generating knockins and knockouts.We have improved the efficacy of PGC isolation and culturing, and were able to generate genetically manipulated cultured PGCs that can colonize the gonads of developing zebra finch embryos. Ongoing efforts are geared towards bringing these embryos to hatching and improving the overall efficacy of current protocols. 3) We are developing and testing AAV viral strains for efficient gene delivery into zebra finch cells. This effort includes screening a mutagenized AAV capsid library to identify variants that confer high infectivity in zebra finch tissues. We have performed several rounds of screening through injections into the brain or blood, and identified several candidate isolates for efficient targeting of finch cells. We have also implemented a bar-coded library approach to systematically compare current and new AAV vectors, to obtain more rigorous and quantitative assessments of their transduction efficacy in zebra finch tissues. We expect that with further development, these tools will be useful for studying the function of genes in a vocal learning species.

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Poster

232. Vocalization and Social Behavior in Songbirds I

Location: Hall A

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Program #/Poster #: 232.17/P20

Topic: F.01. Neuroethology

Support: Midwestern University Faculty Startup

Title: GABAergic neurons of vocal- and non-vocal learning hummingbirds

Authors: *C. R. OLSON[1], K. BERNIER[2], S. MACKEY[3];
[1]Physiol., [3]AZCOM, [2]Midwestern Univ., Glendale, AZ

Abstract: GABA is the main inhibitory neurotransmitter in vertebrates and plays fundamental roles in motor and sensory forebrain processing. We identified GABA expression in vocal learning (Anna's and Costa's, *Calypte anna and C. costae*) and non-vocal learning (black-chinned and rufous, *Archilochus alexandri* and *Selophorus rufus*) hummingbirds, and compared it to that of the zebra finch, a well-studied songbird that evolved vocal learning independent of hummingbirds. We used *in situ* hybridization to label inhibitory interneurons with a riboprobe for GAD2, a marker of GABAergic cells that is well described in the finch model. In our hands, GAD2 expression matched that of previous work in the finch, and its expression was present in the brains of all hummingbirds. Similar to patterns in the finch, GAD2 was prominent in the hummingbird forebrain and enriched in striatum, showing regional heterogenicity in inhibitory neural subtypes. The vocal circuitry of vocal learning hummingbirds (confirmed with darkfield imaging) revealed a subpopulation of large-bodied GAD2-positive cells with enriched expression in the hummingbird VA, VLN and VAN (analogs of RA, HVC and LMAN, respectively). However, these larger cells are absent in adjacent neural tissue. Compared to the finch model, GAD2-positive cells appear to have greater prominence in the vocal circuitry of hummingbirds. Yet, while non-vocal learning hummingbirds had GAD2 expression that was broadly similar to that of the *Calypte* sp. and finch, a major difference is that the prominent GAD2-cells of vocal nuclei were absent. These results suggest that enhanced GABAergic signaling is a critical component of vocal production, and suggests that among the hummingbird lineages that possess vocal learning, employing a strategy of enhanced neural inhibition within the vocal circuit is a necessary component.

Disclosures: C.R. Olson: None. **K. Bernier:** None.

Poster

232. Vocalization and Social Behavior in Songbirds I

Location: Hall A

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Topic: F.01. Neuroethology

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Title: Transcriptional regulatory divergence underpinning species-specific learned vocalization in songbirds

Authors: *H. WANG[1], A. SAWAI[1], N. TOJI[2], R. SUGIOKA[1], Y. JI[1], S. HAYASE[1], S. AKAMA[3], J. SESE[3], K. WADA[4];
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Sapporo, Japan; [3]Natl. Inst. of Advanced Industrial Sci. and Technol., Tokyo, Japan; [4]Fac. of Sci., Hokkaido Univ., Sapporo, Hokkaido, Japan

Abstract: The molecular mechanisms underlying species-specific learned behaviors remain unknown. Songbirds acquire their species-specific songs through learning. Here, we adapted two closely related songbird species, the zebra finch, owl finch, and their interspecific F_1 hybrids, as a model system to elucidate the transcriptional regulatory divergence associated with species-specific song. Using genome-wide quantification of gene expression between the two species and comparing this with the allele-specific expression ratio in hybrids, we identified genes in the vocal motor nuclei whose expression is regulated by species divergence in *cis*- versus *trans*-regulation. Divergence in transcriptional regulation altered expression of approximately 10 % of total transcribed genes and was significantly associated with genes which were differentially expressed between the two species. *Trans*-regulatory changes were more prevalent than *cis*- and affected neural functions for synaptic formation and transmission in song nucleus RA. We further identified BDNF as a potential upstream *trans*-mediator in RA. Pharmacological activation of BDNF receptors eliminated species-specific song features of adult zebra finches. These results suggest that divergence in region-specific transcriptional regulations could be a genetic driver for evolution of species-specific learned behaviors.

Disclosures: H. Wang: None. **A. Sawai:** None. **N. Toji:** None. **R. Sugioka:** None. **Y. Ji:** None. **S. Hayase:** None. **S. Akama:** None. **J. Sese:** None. **K. Wada:** None.

Poster

232. Vocalization and Social Behavior in Songbirds I

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Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 232.19/P22

Topic: F.01. Neuroethology

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Title: Fine-tuning birdsong: The role of delta opioid receptors in the development of song structure

Authors: *U. A. SINGH, S. IYENGAR;
Natl. Brain Res. Ctr., Gurgaon, India

Abstract: We were interested in studying the effects of the opioid system on vocalization and vocal learning using male songbirds (zebra finches, *Taenopygia guttata*). Juvenile male zebra finches learn their songs during a sensitive period and have specialized circuits for song learning and production. Earlier studies from our lab have demonstrated the presence of μ- and δ-ORs in areas specialized for vocal learning and vocal motor production in adult male zebra finches. We

have also shown that blocking μ-ORs using naloxone causes a decrease in the number as well as changes in the spectral and temporal features of adult song. To test the role of δ-ORs in song learning, systemic injections of the δ-OR antagonist naltrindole were administered to juvenile male zebra finches (n = 9) from 35 to 45 days post hatch, a short span of 10 days during the sensitive period. A male sibling from the same clutch was administered the vehicle (saline) for controls. We found that blocking δ-ORs during the sensitive period lead to significant decreases in spectral features of the songs of treated birds when they reached adulthood, including pitch, mean frequency and frequency modulation whereas pitch goodness increased significantly. Systemically blocking δ-ORs also led to changes in the temporal features of song, that is, a decrease in syllable duration. However, there were no changes in the number of songs that treated birds produced, compared to controls. Interestingly, neural changes resulting from naltrindole treatment during the sensitive period for vocal learning manifested as a significant increase in the number of DARPP-32-positive medium spiny neurons and an increase in the number of synapses within Area X, a song control nucleus in the basal ganglia. Our results suggest that δ-ORs may play a role in the development of song structure without affecting the motivation to sing. Further, the δ-OR system also appears to play a role in neurogenesis and/or differentiation during the sensitive period in zebra finches. It is also possible that the increase in DARPP-32-positive neurons following naltrindole administration may somehow compensate for blocking δ-ORs during the sensitive period, leading to spectral features of song becoming prematurely stereotyped.

Disclosures: U.A. Singh: None. **S. Iyengar:** None.

Poster

232. Vocalization and Social Behavior in Songbirds I

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Title: Characterizing distinct cell types in HVC of male zebra finches using single-cell RNA sequencing

Authors: *D. P. MERULLO, A. KULKARNI, M. CO, G. KONOPKA, T. F. ROBERTS; Neurosci., UT Southwestern Med. Ctr., Dallas, TX

Abstract: Speech learning depends on vocal imitation of adult tutors, such as parents. Songbirds learn their songs by imitating adult song tutors through a sensorimotor process similar to speech

learning. The cortical region HVC (proper name) is essential for song learning and song production, and it is proposed to function analogously to the human premotor cortex. Although extensively studied, the basic composition of cell types in HVC remains poorly understood. The limitations of current electrophysiological and anatomical techniques have been a major challenge to comprehensive cell-type identification in HVC. To address this problem, here we perform the first high-throughput classification of HVC cell types in adult male zebra finches using single-cell RNA sequencing (scRNA-seq). Additionally, since HVC is a potential site to encode the memory of a tutor song during learning, we used scRNA-seq to examine HVC in juvenile males. We used the 10X Genomics Chromium system to prepare single-nuclei libraries and then sequenced approximately 20,000 nuclei pooled from male adults or juveniles. We grouped nuclei into discrete clusters using the Louvain algorithm. Cluster visualizations were created with Uniform Manifold Approximation and Projection (UMAP). We then assigned functional identities for each cluster based on reliable cell-type markers from avian and mammalian literature. This approach enabled us to characterize GABAergic interneurons, glutamatergic projection neurons and interneurons, oligodendrocytes, and astrocytes. We assessed the expression patterns of several genes associated with speech learning, including *FoxP1*, *FoxP2*, and *Cntnap2*. *FoxP1* is highly expressed in one putative subtype of neurons that project to Area X, a striatal region essential for song learning. *FoxP2* is almost exclusively expressed in only a single subtype of GABAergic neurons. *Cntnap2* is highly expressed in several subtypes of GABAergic neurons and one subtype of glutamatergic neurons. *Cntnap2* is generally expressed in cells that do not contain *FoxP1* or *FoxP2*, which is consistent with reports that *FoxP1* and *FoxP2* repress *Cntnap2*. To further identify all neuronal projection classes, experiments are ongoing to alter gene expression in specific cell types so that these identities can be observed in scRNA-seq data. Additionally, the effects of recent song exposure on the expression of genes related to learning and memory will be examined across cell types. The results to date provide a robust molecular characterization of the diverse cell types in HVC and thereby generate a means to examine the conserved genes and circuits underlying speech production and vocal learning.

Disclosures: D.P. Merullo: None. **A. Kulkarni:** None. **M. Co:** None. **G. Konopka:** None. **T.F. Roberts:** None.

Poster

232. Vocalization and Social Behavior in Songbirds I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 232.21/P24

Topic: F.01. Neuroethology

Title: Expression patterns of CRF-family peptides in the zebra finch brain

Authors: *K. A. AHMADIZADEH, C. OLSON;
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Abstract: The Corticotropin Releasing Family (CRF) family of peptides are an ancient group that are widely expressed through out the central nervous system of all vertebrates. They play key roles in the body's response to stress, and in the brain drive processes that affect memory and learning. Through both the activation of the hypothalamus-pituitary-adrenal (HPA) axis that regulates glucocorticoid actions, and their release from centrally-projecting pathways, they have potential to alter brain function and behavior. With *in situ* hybridization of serial brain sections we report expression patterns of CRF-family genes in the zebra finch, a model for vocal learning. We note the existence of three CRF-family genes in the zebra finch genome by their homology, syntenic relationships across species, and brain expression patterns. CRH1 is the classic trophic hormone that regulates the HPA axis by its release from cells in the paraventricular nucleus, and this role is conserved in the zebra finch. Its expression in other hypothalamic structures and throughout the forebrain suggests multiple neuromodulatory functions other than glucocorticoid regulation. We also report distinct patterns of expression for CRH2 and UCN3 throughout the midbrain and forebrain of the finch. We lack evidence of expression for UCN1 or UCN2 in songbirds, CRF-family genes that are present in the genomes of basal avian lineages and mammals but seemingly absent in the passeriformes. Because stress, broadly defined, can alter vocal learning of the zebra finch this information is a critical step to future studies that aim to delineate the mechanistic basis of how stress affects learning.

Disclosures: K.A. Ahmadizadeh: None. **C. Olson:** None.

Poster

232. Vocalization and Social Behavior in Songbirds I

Location: Hall A

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Topic: F.01. Neuroethology

Support: NSF 143602

Title: Molecular specializations of the songbird motor cortex

Authors: *A. A. NEVUE[1], P. V. LOVELL[2], C. V. MELLO[2];
[1]OHSU, Portland, OR; [2]Dept. of Behavioral Neurosci., Oregon Hlth. and Sci. Univ. Sch. of Med., Portland, OR

Abstract: Neural circuits involved in the acquisition and production of learned vocalizations have been extensively studied in songbirds. Despite marked differences between the mammalian layered cortex and the nucleated pallium of birds, recent evidence has revealed that the human

laryngeal motor cortex (LMC) and the main vocal motor output nucleus of the zebra finch pallium (robust nucleus of the arcopallium, RA) share a remarkable set of molecular markers that together define a suite of molecular specializations that convergently evolved for vocal production. However, the expression of these shared markers has not been well characterized in any species. Here we examined in detail the expression of these markers, using *in situ* hybridization and focused on RA and an adjacent area (dorsal intermediate arcopallium, AId) in adult male zebra finches. Although the organization and function of AId are not well understood, this area is thought to play a general role in motor control, but alternative views suggest broader integrative roles in vocal learning. The results revealed that a subset of convergent LMC/RA markers represent molecular specializations unique to RA (e.g. *SLIT1*, *GABRB3*, *C1QL3*, *NEUROD6*), whereas another subset of markers are shared between RA and AId (e.g. *PVALB*, *GPM6A*, *PCDH17*, *SNCA*). The latter indicates a close relationship between RA and AId and suggests that RA may have evolved as a specialization of AId, which would be consistent with the motor theory of vocal learning origin. We were also able to molecularly define AId in female zebra finches, in two non-vocal learning suboscine species, and in a distantly related vocal learner (Anna's hummingbird), consistent with a general motor role of AId rather than a specific involvement in vocal learning. Overall, our analysis further defines a set of markers that are uniquely shared between the human LMC and songbird RA, representing a putative molecular underpinning for the production of learned vocalizations. These convergent markers provide a foundation for future studies on the molecular basis of vocal learning.

Disclosures: A.A. Nevue: None. **C.V. Mello:** None. **P.V. Lovell:** None.

Poster

233. Vocalization and Social Behavior in Songbirds II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 233.01/P26

Topic: F.01. Neuroethology

Title: Differential contributions of auditory feedback-dependent and independent mechanisms to the maintenance of song acoustic structure in adult zebra finches

Authors: *D. MIZUGUCHI, S. KOJIMA;
Korea Brain Res. Inst. (KBRI), Daegu, Korea, Republic of

Abstract: Although adult songbirds of many species produce stable song throughout their life, removal of auditory feedback by deafening leads to a gradual deterioration of adult song structure. The detailed mechanisms of this adult song plasticity are not yet fully understood, and two major models have been proposed (Brainard and Doupe, 2000). In the first, 'active instruction' model, song deterioration is actively caused by incorrect instructive signals resulting from the wrong evaluation of auditory feedback. In the second, 'passive drift' model, in contrast,

song deterioration is caused by a passive drift in the vocal control system, which is normally corrected by auditory feedback but not after deafening. Although recent studies support the active instruction model, little is known whether the passive drift process also contributes to deafening-induced song deterioration. To directly test this issue, we prevented adult zebra finches from singing immediately after the deafening surgery for blocking the active instruction process but not the passive drift process. By comparing song structure immediately before and after the 2-week period of singing prevention, we found that deafened birds mostly maintain overall structure of their song during the singing-prevention period. Although singing prevention also caused small but significant decreases in the pitch of many harmonic syllables, such changes were not observed in freely-singing deafened birds. Thus, singing prevention almost completely blocked deafening-induced song deterioration, suggesting that such song changes are predominantly caused by a singing-dependent active process with little contribution of the passive drift process. Additionally, the pitch decrements caused by singing prevention but not by deafening indicate that adult song structure can passively change independently of auditory feedback. Indeed, similar pitch decrements were observed in intact-hearing birds with singing prevention. Moreover, we found similar pitch decrements even in relatively old adult birds, which have been reported to exhibit almost no deafening-induced song plasticity. These results suggest that daily singing behavior is necessary for preventing auditory feedback-independent and age-independent changes in song acoustic structure, providing a plausible explanation for why old birds continue to sing many songs even after losing their auditory feedback-dependent song plasticity. Taken together, our results illustrate the differential contributions of auditory feedback-dependent and independent mechanisms to the maintenance of song acoustic structure in adult songbirds.

Disclosures: D. Mizuguchi: None. **S. Kojima:** None.

Poster

233. Vocalization and Social Behavior in Songbirds II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 233.02/P27

Topic: F.01. Neuroethology

Title: Regulation of repetitive syllable sequence by the excitatory and inhibitory balance in the basal ganglia song nucleus in songbirds

Authors: *Y. JI[1], M. SÁNCHEZ-VALPUESTA[1], K. WADA[2];
[1]Life Sci., [2]Sci., Hokkaido Univ., Sapporo, Japan

Abstract: Stuttering is a vocal communication disorder presenting abnormal repetition of vocalization. The onset of stuttering is associated with dysfunction of the cortico-basal ganglia-thalamic circuit. Here, we adapted songbirds as an animal model to examine the contribution of

the basal ganglia nucleus Area X to regulate the repetitive sequence of songs at the cellular level. We induced the specific ablation of GABAergic neurons in bilateral Area X of Bengalese finch *(Lonchura Striata* var. *domestica*) by injecting AAV containing Cre-FLEx system and expressing Diphtheria toxin A (dtA) driven by Dlx promoter. The frequency of syllable repetition significantly increased after injection compared to pre-injection. Such increasing of syllable repetition did not occur in the other Bengalese finch group that were injected AAV which would express dtA driven by the pan-cellular promoter CMV in Area X. The results suggest that the specific ablation of GABAergic neurons in the basal ganglia may induce excitatory/inhibitory imbalance, which in turn, causes dysregulation of syllable repetitive regulation. The results also raise the possibility that abnormal basal ganglia activity may be involved in the development of persistent stuttering neuropathology.

Disclosures: Y. Ji: None. **M. Sánchez-Valpuesta:** None. **K. Wada:** None.

Poster

233. Vocalization and Social Behavior in Songbirds II

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Support: MEXT/JSPS KAKENHI 17H06380 (#4903)
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Title: Social modulation of auditory activity in a songbird VTA/SNc

Authors: *S. YANAGIHARA[1], M. IKEBUCHI[2], C. MORI[1], R. O. TACHIBANA[1], K. OKANOYA[1,2];
[1]Grad. Sch. of Arts and Sci., The Univ. of Tokyo, Tokyo, Japan; [2]RIKEN, Wako-Shi, Japan

Abstract: As in human speech acquisition, social interactions are crucial for vocal learning in songbirds. Juvenile zebra finches listen to a song directly from a tutor, and faithfully imitate its song. On the other hand, passive exposures to a tutor song presented from a speaker in the absence of a live tutor result in a poor imitation. These behavioral studies highlight the facilitative effects of social interactions in vocal learning, but the underlying neural mechanisms remain unclear. In this study, we hypothesized that social interactions with a tutor is rewarding for juvenile, and hearing a tutor song directly from a live tutor enhances the activity of brain reward circuitry in juvenile leading to successful memorization of a tutor song. To test this, we set out to record multiple single neuron activities from midbrain ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) in freely-behaving juvenile zebra finches, and examined whether the social interactions with a tutor modulate the activity of VTA/SNc neurons.

Consistent with previous electrophysiological studies, a group of VTA/SNc neurons exhibited singing-related or movement-related activity. We further found that another group of VTA/SNc neurons exhibited auditory responses to a tutor song presented through a speaker, and that responses were markedly modulated by social context. These neurons showed greater tutor song responses when a juvenile was in the presence of a tutor compared to alone. Furthermore, similar enhanced auditory responses were observed when a juvenile listened to a song from a live tutor. These results suggest that a group of song-responsive VTA/SNc neurons play a role in social facilitation of vocal learning.

Disclosures: S. Yanagihara: None. **M. Ikebuchi:** None. **C. Mori:** None. **R.O. Tachibana:** None. **K. Okanoya:** None.

Poster

233. Vocalization and Social Behavior in Songbirds II

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Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

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Topic: F.01. Neuroethology

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Title: How social interactions affect attention and song perception in juvenile zebra finches during song learning

Authors: *J. KATIC, Y. YAZAKI-SUGIYAMA;
Okinawa Inst. of Sci. and Technol. (OIST) Grad. Univ., Okinawa, Japan

Abstract: Juvenile male zebra finches learn to sing via vocal communications with their adult tutors. Song learning improves through social interactions with tutors, compared to passive listening to recorded tutor (TUT) song playbacks. This suggests that high attention level, induced by social interactions with tutors, enhances song learning. Here we investigated whether social interactions change attention level and how that change affects song learning by recording neuronal activity in the attention control area of the brain, the nucleus locus coeruleus (LC), and in the higher auditory area, the caudomedial nidopallium (NCM), where tutor song memories are thought to be stored. We chronically recorded extracellular, single-unit activity from LC or NCM neurons of freely behaving juvenile zebra finches before and during social interaction with the tutor. LC and NCM neurons were tested for auditory responsiveness to playbacks of several song stimuli, including TUT playbacks, and live tutor singing. LC neurons increased their firing rates during the song playbacks and responded twice as intensely to live tutor singing as to TUT playbacks (36±2.9 spikes/s to playback, 72±3.2 spikes/s to live tutor singing, n=22). Moreover, LC neuronal responses to TUT playbacks increased after hearing hours of live tutor singing (mean response strength before: 1.6±0.4 spikes/s, and after: 4.5±0.9 spikes/s, n=16). Anatomical

analysis showed that LC neurons, which were activated by exposure to live tutor singing, project to the NCM. Like LC neurons, NCM neurons showed higher firing rates to live tutor singing than to TUT playbacks (6±0.9 spikes/s to playback, 11±1.3 spikes/s to live tutor singing, n=24). Moreover, TUT-selective neurons exhibited greater responses to TUT playbacks after hearing hours of live tutor singing (mean response strength before: 0.4 ± 0.2 spikes/s, and after: 4 ± 0.67 spikes/s, n=24), while non-selective neurons showed no significant increase in response to TUT playbacks. To further understand how LC neurons regulate NCM auditory activities, we optogenetically manipulated activities of LC neuron terminals projecting to the NCM during extracellular recording of NCM neurons. Taken together, we suggest that social interactions with tutors modulate neuronal activity of the LC, which affects selective auditory responses of NCM neurons, resulting in tutor song memory formation.

Disclosures: J. Katic: None. **Y. Yazaki-Sugiyama:** None.

Poster

233. Vocalization and Social Behavior in Songbirds II

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Title: Evolutionary mechanisms in the bengalese finch's song: Parallels and implications for the study of human speech

Authors: *M. FARIAS-VIRGENS[1], T. DEACON[3], K. OKANOYA[4], S. A. WHITE[2], E. HUERTA-SANCHEZ[5];
[2]Integrative Biol. & Physiol., [1]Univ. of California Los Angeles, Los Angeles, CA; [3]Univ. of California Berkeley, Berkeley, CA; [4]Univ. of Tokyo and Riken Brain Sci. Inst., Tokyo, Japan; [5]Brown Univ., Providence, RI

Abstract: Birdsong and human speech are socially learned during development and recruit brain structures with similar function and organization. These parallels have motivated the additional search for similar evolutionary pressures leading to vocal learning in songbirds and humans. Our

research uses a songbird system to identify evolutionary processes leading to increased complexity of learned vocal behavior, a key aspect in speech evolution. The Bengalese finch (BF) (*Lonchura striata domestica*) has a remarkably complex song, in which transitions between vocal units are loosely fixed, introducing variability in song sequencing. This vocal complexity evolved during BF's domestication from the white-backed munia (WBM) (*Lonchura striata*). We are using whole-genome sequencing of individuals within the two bird strains (11 BFs and 11 WBMs) and analytical tools from comparative and population genomics to identify genes that are highly differentiated between them. We have calculated commonly used population summary statistics in non-overlapping sliding windows along the genomes and report several regions showing reduced variation in the BF relative to its ancestor, as evidenced by decreased heterozygosity and nucleotide diversity. We are now using likelihood-based diffusion approximations to infer a demographic model shaping BF's genetic variation and estimate the impact of the population bottlenecks during domestication. We also intend to access the relative contributions of selection processes, such as female choice for complex songs, to BF's evolved song complexity. Another important effect we are measuring is the relaxation of sources of evolutionary constraints to song complexity that are commonly found in the wild but absent in the domesticated scenario, such as stress related to finding food or defending from predators and pressures to avoid confusion with cohabitating finch species. Our results have the potential to guide further comparative efforts toward identifying similar patterns of evolutionary change between humans and other primates or hominid lineages (e.g. Denisovans and Neanderthals).

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Poster

233. Vocalization and Social Behavior in Songbirds II

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Title: Long-term change in neural activity of basal ganglia prior to vocal behavior in Java sparrow

Authors: *S. UMEMOTO, S. YANAGIHARA, K. OKANOYA;
Dept. of Life Sciences, Grad. Sch. of Arts and Sci., The Univ. of Tokyo, Tokyo, Japan

Abstract: In addition to female-directed courtship songs, male songbirds also sing undirected songs (US) without extrinsic reward. Due to these differences, songbirds could be a good model to investigate how internal or external reward motivates behavior. During US, the anterior forebrain pathway (AFP) of songbird brain plays an important role in song learning and modifying. In Area X, a nucleus of AFP in basal ganglia, activity of all pallidal and some striatal neurons increases during US (Goldberg et al., 2011; Fee & Goldberg, 2011). Recently studies in mammals revealed that sequential behavior might be motivated by gradual increase of dopamine level in the striatum (Howe et al., 2013). Since Area X receives dopaminergic input from VTA, activity change in Area X neurons during US might reflect internal motivation expressed in physiological state. In the present study, we explored how the neural activity of Area X correlated with vocal behaviors and their pre- and post-periods. We implanted electrode in Area X of 4 male adult Java sparrows (*Lonchura oryzivora*), and recorded neural activity under free-moving condition. We analyzed firing rate change around US bouts, including calls and introductory notes prior to song syllables. To separate effect of vocalization itself, neural activity around US was compared with that of innate trill calls, which has longer duration than other calls and is used for social communication by Java sparrows. Here we found that firing rate of singing-related Area X neuron gradually increased for few seconds before onsets of song bouts. The activity increase started before any vocalizations prior to song such as introductory notes or calls in most neurons. Comparison between neural activity around song bouts and that around calls revealed that the gradual increase of neural activity prior to vocalization occurred more transiently around trill calls than around song bouts. The results suggest that neural activity in Area X before vocalization reflects long-term changes in motivational states, and we are now exploring a neural network including the other area specifically encoding motivational state to sing US.

Disclosures: S. Umemoto: None. **S. Yanagihara:** None. **K. Okanoya:** None.

Poster

233. Vocalization and Social Behavior in Songbirds II

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Topic: F.01. Neuroethology

Support: NSF GRFP

Title: The neural basis of auditory restoration for familiar zebra finch song

Authors: *M. C. BJORING, C. D. MELIZA;
Dept. of Psychology, Univ. of Virginia, Charlottesville, VA

Abstract: Understanding speech in a noisy environment requires an auditory system capable of restoring occluded speech sounds based on word knowledge and contextual cues. This phenomenon, known as phonemic restoration, has been well characterized in humans, but the neural basis of restoration has received less attention. Songbirds make an ideal model for studying auditory restoration at a neuron level because of their acoustically complex vocalizations which are used for tasks like recognition and mate selection in a noisy colony environment. In speech, phonemic restoration is stronger for native than foreign words, and we hypothesize a similar effect for the songs of familiar and unfamiliar birds. To test this, we modified established paradigms for inducing phonemic restoration of speech to zebra finch song and housed subjects in different social groups, which created different sets of familiar and unfamiliar song. We tested the ability of zebra finches to perform auditory restoration using an operant task. We then analyzed the difference in single-unit responses to identify a neural signature for the restoration of familiar vocalizations. We predict that this signature of restoration will emerge within the auditory processing pathway at a site that merges ascending and descending information, and we have identified the caudal mesopallium (CM) and the caudomedial nidopallium (NCM), both secondary cortical-like auditory areas in the avian auditory system, as potential candidates.

Disclosures: M.C. Bjoring: None. **C.D. Meliza:** None.

Poster

233. Vocalization and Social Behavior in Songbirds II

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ANPCyT, Argentina

Title: Synchronous effects in cortical neurons of songbirds

Authors: *A. AMADOR, S. BOARI, G. B. MINDLIN;
Physics, Univ. of Buenos Aires, Buenos Aires, Argentina

Abstract: How vocal communication signals are represented in the cortex is a major challenge for behavioral neuroscience. Beyond a descriptive code, it is relevant to unveil the dynamical mechanism responsible for the neural representation of auditory stimuli. In this work, we report evidence of massive synchronous neural activity in cortical neurons of songbirds in response to auditory playback of the bird´s own song. These neurons are found in a brain region where sensorimotor integration occurs, and are activated at specific temporal instances of the song.

Disclosures: **A. Amador:** None. **S. Boari:** None. **G.B. Mindlin:** None.

Poster

233. Vocalization and Social Behavior in Songbirds II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 233.09/P34

Topic: F.01. Neuroethology

Support: NSF IOS CAREER 1453084

Title: From perception to action: Sensory cortices and motor pathways underlying female mate choice

Authors: ***J. F. PRATHER**[1], K. S. LAWLEY[3], N. A. STILL[2], J. L. DUNNING[4];
[1]Zoology and Physiol., [2]Dept Zoology and Physiol., Univ. of Wyoming, Laramie, WY; [3]Dept. of Vet. Integrative Biosci., Texas A&M Univ., College Station, TX; [4]Phamacology, Vanderbilt Univ., Nashville, TN

Abstract: Females of many species use male courtship displays as a proxy of male fitness to inform decisions of mate choice. Female mate choice has been studied extensively in songbirds in which females identify males based on their songs, evaluate the quality of those songs, and use that information to guide their mate selection. Song plays a central role in that choice, as female songbirds will exhibit copulatory behaviors (i.e. copulation solicitation displays (CSDs) and calls) in response to songs played through a speaker, even when no male is physically present. Studies of female response to song have implicated auditory cortical regions such as the caudal mesopallium (CM) and the caudal nidopallium (NC) in perception of song quality and the associated influence on production of copulatory behaviors. Here we are employing a combination of excitotoxic lesioning, anterograde and retrograde pathway tracing, and optogenetic stimulation to investigate: 1) the role of those cortical areas in song evaluation and mate choice, 2) the connectivity of those areas with other cortical sites and motor sites that underlie production of courtship behaviors, and 3) the role of specific pathways in specific aspects of song evaluation and mate selection. Our preliminary results from lesion studies demonstrate roles for each of CM and NC in female evaluation of song quality. In ongoing experiments, we are using optogenetic stimulation to further investigate the role of activity in those sites. Pathway tracing data reveal projections from each of those cortical sites to areas implicated in behavioral motivation and production of courtship behaviors, demonstrating possible pathways through which sensory perception may influence motor activation. Future experiments will seek to disambiguate the degree to which activity in specific pathways contributes to sensory perception versus motor activation, and thus the degree to which activity in those brain regions contributes to specific aspects of song evaluation and mate selection.

Disclosures: **J.F. Prather:** None. **K.S. Lawley:** None. **N.A. Still:** None. **J.L. Dunning:** None.

Poster

233. Vocalization and Social Behavior in Songbirds II

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Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

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Topic: F.01. Neuroethology

Support: Department of Biotechnology India BT/HRD/35/02/2006
Scientific and Engineering Research Board EMR/2015/000829

Title: Analyzing the role of distance and feedback from the female on zebra finch courtship song

Authors: H. SURI[1], ***R. RAJAN**[2];
[1]Univ. of Michigan, Ann Arbor, MI; [2]Biol., Indian Inst. of Sci. Educ. and Res. Pune, Pune, India

Abstract: Vocal communication signals are influenced by the distance from a receiver. Many animals (and humans) increase the amplitude of their vocalizations when addressing a distant receiver. However, whether and how distance affects other features of complex learned motor sequences remains poorly understood. Here, we addressed this question by recording and analyzing the songs and visual displays of the adult male zebra finch while varying the distance from a female bird. We found that male birds produced fewer high-intensity courtship songs (songs + characteristic visual displays) as distance from a female increased. Further, our preliminary analyses showed that some features of courtship songs (number of introductory notes and first motif duration) did not change with distance, while other features (number of motifs per bout) changed with distance. We also measured song amplitude using 3 different methods namely, a fixed microphone in the cage, a backpack mounted microphone and a head-implanted microphone. The latter two methods measured amplitude independent of the position of the bird within the cage. Our preliminary analyses showed that song amplitude did not change with increasing distance from the female. However, song amplitude showed random changes between sessions potentially masking changes due to distance. Finally, with increasing distance, we also found that female birds produced fewer responses to male songs and males produced shorter songs when females did not respond. Overall, these results show that distance from a female influences certain characteristics of courtship songs possibly through altered feedback from the female.

Disclosures: **H. Suri:** None. **R. Rajan:** None.

Poster

233. Vocalization and Social Behavior in Songbirds II

Location: Hall A

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Topic: F.01. Neuroethology

Support: NSF IOS 0917918

Title: Shared preference for variations of duet songs between female and male plain-tailed wrens

Authors: N. F. DAY[1], P. RIVERA-PARRA[2], E. S. FORTUNE[3], ***M. J. COLEMAN**[4]; [1]Integrative Biol. & Physiol., Univ. of California Los Angeles, Los Angeles, CA; [2]Dept. de Biología, Escuela Politécnica Nacional, Quito, Ecuador; [3]New Jersey Inst. of Technol., Newark, NJ; [4]Claremont McKenna, Pitzer and Scripps Colleges, Claremont, CA

Abstract: Plain-tailed wrens (*Pheugopedius euophrys*) sing duets that vary from motif to motif within a single bout. These changes in motifs can include the time varying frequency of both female and male syllables, and in the sequencing of syllables. Some of the changes are difficult for human listeners to perceive, but others result in pronounced changes in the cadence and perceived pitch of the duets. The meaning of this variation for males and females in social behavior is not known. Further, the sources of this variability in the motor control systems of females and males is not known.
We examined the responses of HVC neurons in urethane-anesthetized plain-tailed wrens to playbacks of multiple of duet variants. Duets were recorded while the birds were in their home territories in stands of Chusquea bamboo on the slopes of the Andes in Ecuador. Pairs were captured using mist nets, and additional duets recorded while in captivity. Both birds were anesthetized with urethane and recordings made from HVC to playbacks of previously recorded duets and other sounds. HVC neurons in males and females generally respond differently to the same duet song stimulus. In females, HVC neurons responded roughly equally to female and male syllables in duets, whereas in males, HVC neurons responded more strongly during female syllables. Despite these differences, the overall strength of response to a duet variant, as measured using Z statistics, were correlated between males and females. In other words, if neurons in the female HVC responded preferentially to a particular duet variant, neurons in the HVC of the male also preferred that duet. These data suggest a shared preference between the partners for the acoustic parameters of specific duet performances.

Disclosures: M.J. Coleman: None. **N.F. Day:** None. **E.S. Fortune:** None. **P. Rivera-Parra:** None.

Poster

233. Vocalization and Social Behavior in Songbirds II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 233.12/P37

Topic: F.01. Neuroethology

Title: Dynamic feedback between song development and motor behavior in zebra finches

Authors: *J. HYLAND BRUNO;
Columbia Univ., New York, NY

Abstract: In addition to exposure to a model, vocal learning requires social interactions between a pupil and a tutor. To what extent are pupils actors in their own vocal development? Here we used an operant song tutoring system to study how the motor interactions of juvenile zebra finches with a simulated tutor track with the progression of song learning. We trained juvenile male zebra finches on operant song playbacks, and recorded singing behavior continuously during development. By only reinforcing the first 10 key presses on a schedule of two daily quotas, we allowed birds limited exposure to songs. Keys were thus available but inactive at most times of the day; birds experienced no negative consequences for pressing keys beyond the playback quota. We monitored key presses throughout development, and interpreted this activity as a proxy for motivation to hear songs. We observed strong fluctuations in demand for song playbacks during development. Interestingly, these fluctuations were associated with specific vocal changes. In particular, epochs of quiescence (decreased key pressing) as well as dramatic changes in a bird's mode of interacting with keys (e.g., sudden appearance of key press bursts during unreinforced windows) coincided with periods of vocal instability (e.g., emergence of a new song syllable type) or stabilization (e.g., syllable crystallization, elimination of syllable sequence variability). Our findings show that abrupt changes in song structure are often accompanied by equally abrupt changes in patterns of motor behavior associated with the triggering of operant song playbacks. These correlations point to the possibility of bidirectional feedback between developmental vocal changes and motivation to engage in social interactions. These pivotal transitions in song and motor development are likely driven by physiological and molecular changes within the song system, which we aim to explore in future studies.

Disclosures: J. Hyland Bruno: None.

Poster

233. Vocalization and Social Behavior in Songbirds II

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Topic: F.01. Neuroethology

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Simons Foundation Grant 542977SPI

Title: The role of thalamus in maintenance and patterning of persistent cortical activity

Authors: *N. NIKBAKHT, M. S. FEE;
Brain & Cog Sci. / McGovern Inst. for Brain Sci., MIT, Cambridge, MA

Abstract: Performing learned behaviors requires animals to produce precisely timed motor sequences. Birdsong is an excellent natural example of a complex, learned (not innately programmed) and precisely timed motor behavior. Here, using the songbird zebra finch as a model organism, we seek to understand how learned sequences of cortical activity that encode a behavioral "subroutine", such as a song syllable, are controlled and maintained by thalamic input. Birdsong is controlled by a set of brain regions that have evolved to exclusively control singing behavior. Among these, an anterior thalamic region called Uva (nucleus uvaeformis), which is part of a brainstem-thalamocortical feedback loop, is necessary for adult song production. Despite its importance in song production, single unit recordings from Uva have never been reported and as a consequence, the exact cellular and circuit-level function of this nucleus is unknown. We developed a lightweight (~1 g) microdrive and with it performed juxtacellular recordings in adult singing birds. We report, for the first time, single unit recordings from Uva in singing birds and find neurons that primarily burst prior to syllable onsets and other neurons that fire tonically with elevated rates of action potentials throughout the song motif. We hypothesize that Uva actively gates cortical output during song and could be involved in the patterning of syllable order in the learned song. The preponderance of neurons in Uva that burst prior to syllable onsets, and not at other times in the song, is more consistent with a model in which the brainstem-thalamocortical feedback loop acts at the syllable time-scale (~100 ms) and is less consistent with a recent model in which the brainstem-thalamocortical feedback loop acts at fast time-scale (~10 ms) to generate sequences within the song premotor nucleus HVC. Furthermore, the population of tonically active Uva neurons may provide excitatory tone necessary for sequence generation within HVC.

Disclosures: **N. Nikbakht:** None. **M.S. Fee:** None.

Poster

233. Vocalization and Social Behavior in Songbirds II

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Topic: F.01. Neuroethology

Support: Picker ISI Research grant

Title: Attentive listening behavior predicts vocal imitation

Authors: F. FERNANDEZ, M. INSERRA, O. AJALA, M. LANDSTROM, *W.-C. LIU; Colgate Univ., Hamilton, NY

Abstract: Birdsong learning is a protracted developmental process that occurs under a restricted sensitive period, as the juveniles first hear and memorize the adult tutor song during the sensory learning phase. During the later sensorimotor phase, the juveniles produce vocal motor output to match earlier memorized tutor song through enforcement learning. According to this model, passive perception of tutor song during the early sensitive period is sufficient for tutor song acquisition. Using a novel movement-tracking device, here we identify an active, attentive listening behavior in the male juvenile zebra finch (Taeniopygia guttata) which occurs only during the late stage of the sensitive period, as subsong transforms to the imitated plastic song. This listening behavior is characterized by active, immediate approaching toward the singing adult tutor, followed by a few seconds of total silence. Siblings of the same clutch also show individual specific approaching behavior and this idiosyncratic behavior predicts precise vocal imitation. Moreover, administration of dopamine agonist and antagonist both significantly affect the attentive approaching behavior. We suggest that attentive listening behavior that occurs during the sensorimotor learning phase is critical for fine tuning the imitation of syllable structures of the tutor song.

Disclosures: W. Liu: None. **F. Fernandez:** None. **M. Inserra:** None. **O. Ajala:** None. **M. Landstrom:** None.

Poster

233. Vocalization and Social Behavior in Songbirds II

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Title: Delayed auditory feedback interferes with song initiation in zebra finches

Authors: *G. FETTERMAN[1,2], D. MARGOLIASH[1,2,3];
[2]Committee on Neurobio., [3]Dept. of Organismal Biol. and Anat., [1]Univ. of Chicago, Chicago, IL

Abstract: The role of auditory feedback in production and maintenance of learned vocalizations is an active area of research. Paralleling observations in humans, delayed auditory feedback (DAF) induces changes in zebra finch singing including dropped syllables and a perseveration on syllables that resembles stuttering. Previous studies have only reported behavioral changes arising after relatively long periods of feedback perturbation - many hours for conditioned feedback or days to weeks for distorted or delayed auditory feedback and deafening. We investigated the behavioral effects of DAF at shorter timescales, between 4 and 24 hours, comparing them to baseline recordings on the preceding day. Sensitive accelerometers were implanted onto the skulls of male zebra finches, allowing veridical recordings of singing via bone conduction during continuous DAF (cDAF) perturbations. Recorded song from 6 birds was manually labeled and automatically parsed into larger structural features, including motifs (rigidly-determined sequences of syllables), bouts (motifs grouped closely in time, usually preceded by repeated introductory notes), and phrases (bouts grouped in time). Within the first 4 hours of exposure to cDAF (and possibly more rapidly), we observed striking changes in singing behavior. A compelling result was the appearance of runs of introductory notes not followed by song motifs, a phenomenon we call “zero motif bouts.” These are uncommon in normal song (absent in 4 of the 6 birds, and rare in the other 2), but under DAF their prevalence increased dramatically (35 ± 24% of bouts, range 5-70%, N = 6 birds). Single-bout phrases appeared much more frequently under DAF. Later bouts in a phrase also were preceded by significantly more introductory notes, and for all bouts the inter-introductory-note interval increased significantly as well. These observations all support a simple model for the initial effects of DAF on singing: initiation of song is impaired, possibly involving disruption of preparatory activity attendant upon vocalizing introductory notes. These results may be a behavioral manifestation of rapid changes in intrinsic electrophysiological properties that have recently been observed in song system neurons projecting to the basal ganglia in birds exposed to short periods of cDAF. This suggests that network structure may underlie auditory feedback responses in the song system, which are normally suppressed during singing in zebra finches. We are exploring this possibility.

Disclosures: G. Fetterman: None. **D. Margoliash:** None.

Poster

233. Vocalization and Social Behavior in Songbirds II

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Topic: F.01. Neuroethology

Support: NIH Grant R01NS099288
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Title: Dopamine action in a sensorimotor region

Authors: *A. A. MERCER[1], R. D. MOONEY[2];
[1]Duke Univ., Durham, NC; [2]Duke Univ. Dept. of Neurobio., Durham, NC

Abstract: Learning by imitation is central to human behavior, giving rise to speech, language, and many other important behaviors. Just as in children learning to speak, male juvenile zebra finches selectively memorize and copy songs produced by a conspecific adult tutor, which requires a learning mechanism that integrates social and auditory information provided by a singing tutor. The forebrain sensorimotor nucleus HVC is likely a crucial site where auditory and social cues are integrated to form a memory of the tutor song. HVC receives auditory cortical inputs that convey information about tutor song and input from dopamine (DA)-releasing midbrain neurons that are activated by the presence of a live, singing tutor but not by auditory stimulation alone. Our goal is to understand the effects of DA, which signals social context, on various cells and synapses in HVC, paying particular attention to auditory synapses onto HVC cells. To determine how DA affects intrinsic properties of HVC projection neurons (PNs), we made whole cell patch clamp recordings from identified striatal-projecting and premotor HVC cells in acute brain slices. Bath-application of DA caused an increase in depolarization-induced firing rates and a more depolarized resting membrane potential in both HVC PN types, suggesting that DA increases excitability in these cells. To test how synaptic currents are affected by DA, we electrically stimulated HVC afferents while blocking inhibitory transmission, which likely activates auditory inputs, among others. In HVC PNs, this stimulation induces EPSCs that are multi-peaked in some cells, possibly due to local recurrent activity. Bath-applied DA enhances these EPSCs, increasing the total charge transfer. Taken together, these findings indicate that DA, which signals social context, can alter cellular and synaptic properties of HVC PNs. Future studies will use optogenetics to isolate auditory inputs to HVC and determine how DA acts on these synapses, testing the hypothesis that coincident social context (DA) and auditory information potentiates auditory synapses in HVC.

Disclosures: A.A. Mercer: None. **R.D. Mooney:** None.

Poster

233. Vocalization and Social Behavior in Songbirds II

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Support: NIH R01NS099288
NSF 1354962

Title: Identifying LMAN-dependent and learning-related spectrotemporal patterns in juvenile zebra finch song syllables

Authors: *S. N. BRUDNER[1], J. GOFFINET[2], J. M. PEARSON[2], R. MOONEY[1];
[1]Neurobio., [2]Biostatistics and Bioinformatics, Duke Univ., Durham, NC

Abstract: Children learn speech and other crucial skills by imitating adult behaviors. Learning by imitation requires not only a suitable model but also engaging in appropriate practice. An influential idea is that to imitate an adult target, juveniles must: (1) produce sufficiently variable behavior to sample both target-similar and target-dissimilar actions, and (2) bias the distribution of their behavior towards increasingly target-similar actions. We do not understand how the juvenile brain implements and coordinates these processes, although neurological evidence suggests that cortico-basal ganglia (CBG) circuits support successful practice. Zebra finch song learning, a primary model for studying juvenile imitative learning, requires a song-dedicated CBG circuit that influences practice singing through a cortical nucleus called LMAN. In addition to increasing song variability in juveniles and adults, LMAN can alter song acoustic distributions to avoid pitch-contingent punishment in adults. This observation motivates the hypothesis that LMANadaptively biases juvenile song towards the imitation target.Testing this hypothesis requires identifying the spectrotemoporal changes in song structure underlying the transition from immature to mature song, and assessing dependence of more mature spectrotemporal patterns on LMAN activity as they emerge. Here, we deploy a tool from unsupervised machine learning, the variational autoencoder, to generate informative fixed-length vector descriptions of song syllable spectrotemporal patterns. We leverage this descriptive framework to model changes in spectrotemporal patterning during development. Finally, we repeatedly, transiently inactivate LMAN in learning juveniles to compare the spectrotemporal patterns introduced acutely by LMAN premotor activity and the ongoing systematic changes to spectrotemporal patterning that result from learning. In particular, we assess whether LMAN induces an acute bias in modal song characteristics, and whether such a bias predicts the trajectory the juvenile will take towards his target song. This experiment may have implications for the premotor function of cortico-basal ganglia circuits in juvenile imitative learning more generally.

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Poster

233. Vocalization and Social Behavior in Songbirds II

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HCA-A-1704-01747 Chan Zuckerberg Initiative (GK)
NIH R01 MH102603 (GK)

Title: Knockdown of the autism-associated gene FoxP1 in corticostriatal song neurons blocks the cultural transmission of birdsong

Authors: F. GARCIA-OSCOS[1], V. K. DALIPARTHI[3], M. CO[4], F. AYHAN[2], T. KOCH[2], D. P. MERULLO[4], H. PANCHOLI[2], J. E. HOLDWAY[2], A. KULKARNI[2], G. KONOPKA[2], *T. F. ROBERTS[2];
[1]UT Southwestern Med. Ctr., Dallas, TX; [2]UT Southwestern Med. Ctr., UT Southwestern Medical Center, TX; [4]Neurosci., [3]Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: Autism spectrum disorders (ASD) are characterized by impaired social communication and restricted or stereotyped behaviors and interests. Many of the social and communicative behaviors disrupted in ASD are initially learned by interacting with and emulating the behavior of parents, teachers, and other more experienced individuals. These complex forms of social communication are difficult to study in traditional laboratory animal models, which has limited our understanding of how distinct disruptions in neural circuits directly impair social communication. Here we examine how knockdown of the ASD-linked gene *FoxP1* (FP1-KD) influences the cultural transmission of song in zebra finches, a songbird species that learns its courtship song during a developmental sensitive period by memorizing and then copying the song of an adult 'tutor'. Haploinsufficiency of *FOXP1* causes a neurodevelopmental disorder characterized by disruptions in expressive speech and language, ASD-related traits, and intellectual disability. The FoxP1 transcription factor is strongly expressed in the song premotor nucleus HVC, a cortical region necessary for learning from social experiences with a tutor in juvenile birds and for producing learned song in adulthood. Using anatomical tracing, single-cell RNA sequencing, and imaging approaches, we show that FoxP1 is strongly expressed and can be virally knocked down in HVC corticostriatal neurons (HVC_X neurons: HVC neurons projecting to the striatopallidal nucleus Area X). We find that FP1-KD in juvenile birds (35dph) disrupts their ability to memorize the song of a tutor, even

when they are given extensive time to learn from their tutor (housed with tutor from 45-65dph). However, if the song was memorized beforehand, FP1-KD does not interfere with the juvenile's ability to remember and learn to produce the song. We find that FP1-KD leads to an increased excitation-inhibition (E-I) ratio in HVCx neurons, causing hyperexcitability and an increase in spontaneous glutamate release onto these neurons. Moreover, we find that birds tutored following FP1-KD sing simple, repetitive songs that are qualitatively different from the adult songs of normal or untutored birds. Together, these findings indicate that FP1-KD selectively impairs learning from salient social experiences that guide song learning and implicate a corticostriatal circuit in this formative stage of social development.

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Poster

233. Vocalization and Social Behavior in Songbirds II

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Topic: F.01. Neuroethology

Support: NICHD Grant R15HD085143
Brachman Hoffman Fellowship

Title: Older and newer memories activate distinct regions in the auditory forebrain of songbirds raised with two tutors

Authors: *P. ARYA[1], S. PETKOVA[3], P. P. KULKARNI[4], N. H. KOLODNY[2], S. M. H. GOBES[1];
[1]Neurosci., [2]Chem., Wellesley Col., Wellesley, MA; [3]Dept. of Pschyiatry and Behavioral Neurosci., Univ. of California, Davis, Davis, CA; [4]Psychology, Northeastern Univ. Dept. of Psychology, Boston, MA

Abstract: Sensory experiences during early development shape cognitive functions such as language acquisition in humans or song acquisition in songbirds (Hensch, T. K., 2005, Sanes, D. H. & Bao, S., 2009). Like humans, zebra finches (*Taeniopygia guttata*) must learn their vocalizations from another conspecific adult during a critical period. Zebra finch males acquire an auditory memory of the song of an adult tutor early in development. Later in development, through vocal practice and trial and error learning, they develop their own unique song. When exposed to two different tutors sequentially, zebra finches are able to learn and imitate the song from a second tutor (reviewed by: Gobes, Jennings, and Meada, *in press*). However, we do not know how plasticity in the neural substrate for song learning can support sequential memory

formation for two song models. In this study, we performed functional MRI to trace changes in the neural substrate for tutor song memory throughout development. Juvenile zebra finches were exposed to two tutors: the first tutor until 30 days post hatching (dph), which is during the sensory acquisition phase, and the second tutor at 55-65 dph, during the sensory-motor learning phase. The BOLD fMRI signal of anesthetized birds in response to songs from both tutors was acquired at 55dph just before the introduction of the second tutor, and at 90dph when the birds had developed their own song. In 55-day old juveniles, we found neural activation for songs from the first tutor in the left caudolateral nidopallium (NCL), and a bilateral response in the primary auditory region Field L in response to the song of the second—still unfamiliar— tutor. At the end of development (90dph), we found left-dominant brain activation in response to songs from the second tutor in secondary auditory regions. In response to the song from the first tutor, activation was left dominant in the primary auditory region Field L. Our data show that distinct parts of auditory cortex are activated by playback of songs from two different tutors, suggesting that older and newer memories may be processed in different brain regions.

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Poster

233. Vocalization and Social Behavior in Songbirds II

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Support: NIH RO1 NS108424
TCU IS 66038

Title: Respiratory antecedents of song initiation and termination

Authors: J. DUKES, S. WHARTON, B. VO, A. B. DUPLECHAIN, A. M. FARIAS, ***B. G. COOPER**;
Texas Christian Univ., Fort Worth, TX

Abstract: Exploration of the neural control of learned motor sequences has revealed preparatory activity that is likely related to motor planning. Vocal learning in songbirds is an example of a sequential motor behavior that is first and foremost a respiratory motor act; elucidating the neural control of song motor planning requires investigation of respiratory antecedents of song initiation and termination. We have found that zebra finches (*Taeniopygia guttata*) show preparatory respiratory patterns starting ~1 s before song and that there are distinct post-song respiratory changes. We compared respiratory patterns between zebra finches and Bengalese finches (*Lonchura striata domestica*) prior to, during, and following song bouts. In both species,

compared to quiet respiration surrounding song, song respiratory patterns are generated with higher amplitude, faster tempo, and ~70% of the respiratory cycle is in the expiratory phase. In directed (female present) and undirected (isolation) singing contexts, both species show a change in the proportion time spent inhaling ~1 s prior to song. Compared to Bengalese finches, zebra finches show greater acceleration of the respiratory rhythm when singing undirected song. During song, zebra finches are more likely to produce longer duration expirations than Bengalese finches. Mini-breaths are typically silent and function to replenish the air supply and prepare for the upcoming vocalization. Zebra finches take shorter duration, larger amplitude mini-breaths than Bengalese finches during song. These respiratory differences could be related to differences in motor planning to execute the upcoming song vocalization and may explain why zebra finches show greater acceleration pre-song respiratory rhythm compared to Bengalese finches. Following song, only zebra finches show systematic changes in respiratory patterns; in this species they spend a greater proportion of the respiratory cycle in the expiratory phase for one second after song. This is likely related to the fact that zebra finches hyperventilate during song, which may induce song termination. These results illustrate that changes in respiratory patterns prior to song may reflect the motor planning for the upcoming song production, species differences in preparatory motor activity could be related to the degree to which the planning is required, and, last, song termination is likely dictated by respiratory demands. Therefore, the neural control of song likely utilizes proprioceptive feedback from respiratory motor systems as well as chemo-sensitive feedback to regulate the initiation, production, and termination of song.

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Poster

233. Vocalization and Social Behavior in Songbirds II

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Topic: F.01. Neuroethology

Support: NSF Grant 1557499

Title: A neural circuit for more than just singing in the female songbird

Authors: ***J. BURKE**[1], C. MESSIER[1], J. JARMULA[1], M. WILD[2], M. SCHMIDT[1];
[1]Biol., Univ. of Pennsylvania, Philadelphia, PA; [2]Univ. of Aukland, Aukland, New Zealand

Abstract: The songbird, with its unique and complex vocal abilities, has become a key model system for understanding vocal control. Since the mid-1970s, much progress has been made in identifying a neural circuit of interconnected cortical and subcortical nuclei that is responsible for the control, learning and modulation of song. The approach used to discover this “song

system" was biased, however, for identifying a circuit that innervated the vocal musculature. We argue that the exclusive focus on singing, and primarily in species where males lack postural displays, has biased the field toward assuming that the "song system" is exclusively involved in vocal control. Recent phylogenetic analyses suggest that song is the ancestral trait for passerines and indeed there exist many species of birds in which the females also sing. In fact, in some tropical species, the females can produce song at levels that rival that observed in the male. The presence of song control nuclei in females therefore suggests that the song system might be associated with the ability of females to sing. Interestingly, there are many species in which the female does not sing yet the female brain shows many of the same song control nuclei as the male. One such example is the brown-headed cowbird (*Molothrus Ater*), an icterid species in which the males sing to females during the breeding season and females respond by producing a posture known as the copulation solicitation display (CSD). Using the cowbird as a model, we propose that in the nonsinging female songbird, the "song system" may be modulating the postural response to male song. While a scattering of studies have described the existence of various song control nuclei in female songbirds, there is no single study showing full connectivity of the song system in the female songbird, let alone in a species where the female does not sing. In this study we use a combination of standard Nissl histology and targeted tracer injections to map out known connectivity of the song system, from nuclei in the forebrain to motoneuron pools in the spinal cord. Our findings are consistent with the notion that female songbirds, in species such as the cowbird that do not sing, contain the same overall connectivity pattern of the song system as has been observed in males.

Disclosures: J. Burke: None. **C. Messier:** None. **J. Jarmula:** None. **M. Wild:** None. **M. Schmidt:** None.

Poster

233. Vocalization and Social Behavior in Songbirds II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 233.22/Q5

Topic: F.01. Neuroethology

Support: NIH Grant DC014432
NIH Grant GM120464
NSF Graduate Research Fellowship DGE-1448072

Title: The developmental regulation of sex-linked genes in sexually dimorphic song nucleus RA

Authors: *S. R. FRIEDRICH, C. V. MELLO;
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Abstract: In many bird species including the zebra finch (*Taeniopygia guttata*), birdsong is sexually dimorphic in both behavior and the underlying brain anatomy. The development of song in males but not females is mirrored by marked dimorphism in song system development, including gross differences in song nucleus RA (robust nucleus of the arcopallium). At 20 dph (days post-hatch), before juveniles have begun to sing, RA is similar in volume and cell density between males and females. After 20 dph, RA continues to grow in males but regresses in females. While this progression has been shown to be dependent on sex steroids, the genetic mechanisms driving the development of this sexual dimorphism remain largely unknown. Due to the differential copy number between sexes and apparent lack of complete, chromosome wide dosage compensation in zebra finches, genes located on sex chromosome Z are interesting candidates to explore this question. Here, we used microarray data and *in situ* hybridizations to identify sex chromosome genes that are differentially regulated in RA. Specifically, we compared the expression of sex-linked genes in males and females before and after RA diverges in development. We observed a broad range of differential regulation across sexes and ages for genes representing a variety of molecular functions and pathways. These findings provide insights into how sex-linked genes may contribute to the development of a highly dimorphic trait.

Disclosures: S.R. Friedrich: None. **C.V. Mello:** None.

Poster

233. Vocalization and Social Behavior in Songbirds II

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Program #/Poster #: 233.23/Q6

Topic: F.01. Neuroethology

Support: NINDS 1 R01 NS104008-01
NSF award DGE-1632976

Title: Effects of inactivation of the periaqueductal gray on song production in testosterone-treated male canaries (Serinus canaria)

Authors: *C. M. HAAKENSON[1], F. N. MADISON[2], N. H. PRIOR[1], G. F. BALL[1];
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Abstract: Male canaries (*Serinus canaria*) display seasonal changes in the motivation to sing which have been found to be dependent on the action of testosterone (T). During the breeding season when T is high, males sing at a higher rate compared to males with low T. The effect of T on song rate is known to be mediated by the medial preoptic nucleus (POM); however, it is unclear how T-signaling in POM impacts song production. One potential mechanism is via modulation of dopaminergic input into song control nuclei by the periaqueductal gray (PAG). In

order to test the role of PAG in T-mediated song production, we treated male canaries with T implants and implanted guide cannula targeting the PAG. Through these guide cannula, we transiently inactivated PAG with injections of the GABAa agonist, muscimol. Each bird received both muscimol and saline with a 48-hour washout period between treatments. The order of injection type was randomized and counterbalanced between individuals. Preliminary data from this study suggests that muscimol in the PAG increases the latency to sing post-injection, decreases song rate and overall time spent singing in the hour following the first song post-injection. These results are consistent with the hypothesis that T-induced singing is regulated via dopamine action in the PAG.

Disclosures: C.M. Haakenson: None. **F.N. Madison:** None. **N.H. Prior:** None. **G.F. Ball:** None.

Poster

233. Vocalization and Social Behavior in Songbirds II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 233.24/Q7

Topic: F.01. Neuroethology

Support: Mathers Foundation
Simons Collaboration on the Global Brain

Title: Neural correlations in a cortical circuit that generates behavioral variability

Authors: *G. F. LYNCH, M. S. FEE;
McGovern Inst. for Brain Res. / Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: Despite the crucial role of variable exploratory behavior in learning, how the brain generates this variability remains poorly understood. Songbirds imitate the song of their tutor through a process of trial-and-error learning, and in the earliest stages of learning they sing highly variable songs . The behavioral variability underlying this process requires the brain region lateral magnocellular nucleus of the anterior nidopallium (LMAN), which is the cortical component of a basal ganglia (BG) thalamocortical loop.
How does LMAN generate behavioral variability? A number of models propose that interconnected neurons within LMAN exhibit chaotic dynamics, thereby producing seemingly random patterns of activity that drive behavioral variability. One such model posits that LMAN is a balanced excitatory-inhibitory (EI) network with uniformly random connectivity, and predicts that pairs of neurons within LMAN would have uncorrelated activity. Another model posits that LMAN may act as an excitable media producing locally propagating waves of activity, and predicts that nearby pairs of neurons would be highly correlated. To test these models and to understand how LMAN actively generates behavioral variability, we built a

miniature lightweight microdrive to simultaneously record from multiple neurons, and with it recorded the simultaneous activity of pairs of single units (N = 25 pairs) in singing juvenile birds (N = 7 birds). We find that the majority of pairs exhibit weak or non-significant firing rate correlations (23/25 pairs). However, some pairs of neurons have extremely large correlations, even after correcting for song-locked activity (2/25 pairs, Pearson correlation > 0.5). The existence of such highly correlated neurons within LMAN is inconsistent with LMAN being a simple balanced EI network with uniformly random connectivity. Intriguingly, even though highly correlated pairs of neurons had small spatial separations (<250um), most of the similarly nearby pairs had no significant correlation (17/25 pairs, distance < 250um). The preponderance of uncorrelated neurons at small distances is also inconsistent with LMAN acting as an excitable medium that supports local wave propagation.

Disclosures: G.F. Lynch: None. **M.S. Fee:** None.

Poster

233. Vocalization and Social Behavior in Songbirds II

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Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 233.25/Q8

Topic: F.01. Neuroethology

Support: NINDS 1 R01 NS104008-01

Title: Intrinsic sex differences in steroid hormone-modulated neuroplasticity in canaries (Serinus canaria)

Authors: *F. N. MADISON[1], C. M. HAAKENSON[2], A. R. WHITAKER[1], A. M. KAWIISO[1], G. F. BALL[3];
[1]Dept. of Psychology, [2]Neurosci. and Cognitive Sci. Program, Dept. of Psychology, [3]Univ. of Maryland, College Park, MD

Abstract: Temperate zone songbirds, such as the canary (*Serinus canaria*), experience marked seasonal plasticity in both song behavior and brain morphology. In males, the increase in daylengths during spring is associated with an increase in gonadal volumes and circulating testosterone. In response to exogenous testosterone, females demonstrate a marked increase in song frequency and quality in addition to male-like changes in brain morphology, namely in the telencephalic nucleus, HVC (acronym is proper name). Although much is known about the relationship between sex steroid hormones and adult brain plasticity in the canary, possible sex differences in testosterone-induced adult neuroplasticity in the song control system is not well understood. We sought to address whether the female canaries are intrinsically less sensitive to the masculinizing effects of testosterone on brain morphology or if females require a longer duration of exposure to experience the same result. Males were castrated and females were

photoregressed and housed in our aviary on short days (8L:16D) for at least 6 weeks until they were photosensitive. Birds were surgically implanted with a 12 mm (10mm packed) testosterone-filled Silastic implant and immediately individually housed in sound attenuated chambers on an 8L:16D light cycle for either 3 or 12 weeks. Brains were extracted, sectioned, and HVC volumes were measured based on Nissl stained sections. Brain sections were also analyzed via immunohistochemistry for doublecortin, a marker for new neurons. We found no significant difference in circulating serum testosterone concentrations in male and female canaries implanted with testosterone for either 3 or 12 weeks. Males demonstrated significantly higher HVC, RA, and Area X volumes at both 3 and 12 weeks. However, the number of doublecortin-ir cells in HVC did not differ in response to treatment at both timepoints. These data suggest that testosterone-driven mechanisms in adult canary neuroplasticity works in a sex-specific manner to mediate marked increases in brain morphology.

Disclosures: F.N. Madison: None. **C.M. Haakenson:** None. **A.R. Whitaker:** None. **A.M. Kawiiso:** None. **G.F. Ball:** None.

Poster

233. Vocalization and Social Behavior in Songbirds II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 233.26/Q9

Topic: F.01. Neuroethology

Support: Picker ISI

Title: Making a move: The effects of flight movement on vocal imitation and juvenile neurogenesis

Authors: *C. M. SENNECA, A. GILMAN, M. CEALIE, M. LANDSTROM, W.-C. LIU; Colgate Univ., Hamilton, NY

Abstract: The motor theory of speech production predicts that the exercise of non-vocal motor control may influence the development or evolution of vocal-motor learning. Here we investigate how flight movement in juvenile songbirds may be associated with vocal imitation capacity during the sensitive period of vocal learning. Using a novel movement tracking device in zebra finches, we show that during the sensorimotor learning phase, the daily rhythm of flight movement in the developing juveniles is correlated with their daily activity of singing practice, as the production of plastic song is often accompanied by frequent flight movement. Additionally, juvenile siblings that have more frequent flight movement tend to perform better imitation of tutor song. The vocal imitation does not correlate with other social factors, such as the close proximity with the father tutor or the interaction among siblings. We show that when limiting the flight movement by wing-clipping at nestling stage, the manipulated birds develop

worse song imitation as adults. We also investigate whether the impaired flight movement is associated with extent of juvenile neurogenesis in song nuclei HVC and Area X.

Disclosures: C.M. Senneca: None. **A. Gilman:** None. **M. Cealie:** None. **M. Landstrom:** None. **W. Liu:** None.

Poster

233. Vocalization and Social Behavior in Songbirds II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 233.27/Q10

Topic: F.01. Neuroethology

Support: FRQNT
NSERC

Title: Experience dependent plasticity in the auditory preferences of female songbirds

Authors: *E. M. WALL[1], S. C. WOOLLEY[2], A. MURADYAN[3];
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Abstract: Vocal communication requires proper interpretation of vocal signals which can depend not only on the content of the signal, but also on an individual's experiences. Female songbirds, such as the zebra finch, are an excellent model for studying auditory perception as they use learned vocal signals (songs) produced by males to identify individuals and choose mates. During development, the auditory system of zebra finches is highly plastic and shaped by early experience and birds reared without exposure to song display poor frequency discrimination and aberrant song preferences. However, the mechanisms underlying experience-dependent adult plasticity and the degree to which it can remedy deficits that result from altered developmental auditory experience are unknown. To address this question, we investigated how adult mating experience affected the formation and direction of female song preferences. Females were either reared with both parents (normally-reared) or with mothers (song-naïve). Then, as adults, females from both rearing conditions were either housed in the same cage as a male (mated) or with another female separated from the male-female pair by an opaque barrier (unmated). Thus, all females received the same auditory exposure to the male's song but differed in their physical and visual interactions. Each set of pairs was filmed upon the first introduction, after one week, and after two weeks of cohabitating to assess pair bonding and mating success. Following this social experience, we tested behavioral responses of females to song using an active choice task. We found that mated females from both rearing conditions heard similar amounts of song, displayed affiliative behaviors indicative of pair bonding, and did not differ in aggression (n=18). Moreover, we found that two-weeks of co-habitation was sufficient for normally-reared females to form strong preferences for the mate's song (n= 9). Surprisingly,

females reared without developmental exposure to song also learned to prefer their mate's song and showed species-typical responses to novel song stimuli (n=9). In contrast, unmated females, regardless of rearing condition, showed no preference for the familiar male's song over an unfamiliar conspecific, indicating that familiarity with the song alone is not sufficient to impact preference (n=35).Thus, courtship and mating interactions appear to strongly influence preference learning and may ameliorate perceptual deficits resulting from impoverished acoustic experience during development. In addition, these data suggest that receiving auditory stimuli demands more than passive listening - it requires dynamic encoding of the signal.

Disclosures: E.M. Wall: None. **S.C. Woolley:** None.

Poster

233. Vocalization and Social Behavior in Songbirds II

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Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

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Topic: F.01. Neuroethology

Support: NIH Grant DC012859
NIH Grant 1S10OD018495
Big Ideas Generator, University of Chicago

Title: Individual specific intrinsic neuronal properties in the bird song system

Authors: A. DAOU, ***D. MARGOLIASH**;
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Abstract: Recent studies highlight the role of neuronal intrinsic properties (IP) in learning processes. We investigated the intrinsic plasticity expressed by HVCx neurons, which provide the song system forebrain projection to the basal ganglia. Across all conditions, whole cell patch slice recordings were made from 370 HVCx in 76 animals. A principal result was that IP were notably similar for recordings of multiple HVCx from the same bird, and yet varied from bird to bird. This was observed in numerous attributes of the raw data (features of spike shapes and spike trains elicited in response to current injections). Control experiments including same-day recordings of pairs of birds using the same ACSF, blocking fast synaptic transmission, blocking specific currents in voltage-clamp recordings, and others ruled out non-specific effects. We modeled these data in a Hodgkin-Huxley framework, demonstrating that the magnitude of five ion currents that are pharmacologically confirmed for HVCx all co-varied among birds. Exhaustive parameter searches (> 2.4 x 10^9 sets of conductance magnitudes per cell) on a subset of cells demonstrated that the manual fits of the HH conductances yielded unbiased results. Given that each bird was represented by a tight cloud of five ion current conductance magnitudes, we represented each bird by the centroid in the 5D space. A second principal result

was the distance in the 5D space between two birds was inversely related to the similarity of the songs of the two birds. We confirmed a prediction of this result, that sibling birds singing essentially the same songs have essentially identical IP (N = 4 pairs of sibs). The contribution of genetics and learning to this result remains to be explored. These results imply that IP is affected by learning but do not identify the time course of those effects. To this end, we demonstrated that IP variance is much higher in individual juvenile birds (learning to sing) than in adults. In adults, we also assessed IP by exposing birds to varying intervals (hours to days) of continuous delayed auditory feedback (cDAF), which rapidly induces changes in song initiation behavior followed by longer-term changes. A third principle result was that there were profound changes in the IP of HVCx in birds exposed to cDAF for days, even in the single bird with only a 4 hr cDAF exposure. There was an approximate logarithmic fit between the degree of scatter in HVCx IP and the duration of cDAF exposure. The results suggest that HVCx IP are regulated rapidly on a motif-by-motif basis during singing. Collectively these results provide new insights into mechanisms of birdsong learning, and mechanisms of regulation of neuronal networks.

Disclosures: D. Margoliash: None. **A. Daou:** None.

Poster

233. Vocalization and Social Behavior in Songbirds II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 233.29/Q12

Topic: F.01. Neuroethology

Support: NIH R01 NS094667
PEW Scholar

Title: Habenula lesions in songbirds cause species-atypical vocalizations

Authors: *A. C. ROESER, J. H. GOLDBERG;
Dept. of Neurobio. and Behavior, Cornell Univ., Ithaca, NY

Abstract: The habenula (Hb) is an evolutionarily conserved part of the limbic system that relays information from ventral pallidum (VP) to dopaminergic (DA) midbrain. In mammals and fish, Hb neurons are activated by aversive stimuli, and human Hb is also associated with psychiatric conditions such as depression and chronic pain. Yet the role of Hb in motor sequence learning remains poorly understood. Here we combine viral tract tracing and behavioral studies to show for the first time that the songbird Hb is important for song learning. First, we found that the Hb links VP to DA midbrain, as in mammals. To test the role of Hb in song learning, we chemically and electrolytically lesioned the Hb in juvenile zebra finches and recorded their vocal development until adulthood. Hb-lesioned birds produced highly unusual vocalizations, including high-pitch notes and species-atypical trills similar to what has previously been

observed in socially isolated birds. These findings demonstrate that 'limbic' circuits, with established roles in hedonic functions and emotional processing are also necessary for a motor sequence learning task like birdsong.

Disclosures: A.C. Roeser: None. **J.H. Goldberg:** None.

Poster

233. Vocalization and Social Behavior in Songbirds II

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Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

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Topic: F.01. Neuroethology

Support: NIH Grant T32DA043469

Title: Assessing the contributions of genetics and sensorimotor learning in a population of forebrain song system neurons

Authors: *N. D. MEDINA[1,2], D. MARGOLIASH[1,2,3];
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Abstract: The understanding of the cellular correlates of learning has largely focused on synaptic changes, potentially overlooking contributions of non-synaptic mechanisms of plasticity such as changes in neuronal intrinsic properties (IPs). Previous results from zebra finches establish a striking relationship between the IP of forebrain neurons and singing behavior. The IPs of HVC basal-ganglia-projecting neurons (HVCx) are very similar within individual birds and different across birds, and the difference in IP between two birds is predictive of their songs' similarity. There are also several reports that HVCx IP are developmentally regulated, including the within-bird similarities which are absent or reduced in juvenile birds. These results motivate several questions, including whether various attributes of singing behavior are related to specific attributes of IPs, how these emerge developmentally, and what are the relative contribution of genetics and song learning to the expression of HVCx IPs in adult birds. Here, we address this third question. We are investigating how genetics affects the expression of IPs. Our approach is to use a counterbalanced design to raise sibling and unrelated birds to sing one of two songs and assess the IPs of the HVCx population. Using a previously established Hodgkin-Huxley model representing the magnitudes of five ion currents known for HVCx neurons, we will estimate conductance values of HVCx neurons from current-clamp slice recordings. HVCx from a given bird will cluster in a restricted region of five-dimensional conductance space. The position of clusters may be constrained by genetics and shifted as a result of song learning. For example, siblings may be closer in conductance space than birds singing similar songs if genetics more directly control IPs, while closely overlapping clusters from birds that sing the same song would strongly suggest that IPs are entirely a product of song learning. The extent to which birds cluster

close to siblings or to similarly tutored conspecifics will provide a readout of the relative contribution of genetics over sensorimotor learning to IPs. These results will elucidate the role of intrinsic plasticity in network function at large and contribute to a more complete understanding of the mechanisms that underlie song learning.

Disclosures: N.D. Medina: None. **D. Margoliash:** None.

Poster

234. Reward, Value, and Decisions

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 234.01/Q14

Topic: G.02. Motivation

Support: KAKENHI 16H04652

Title: Superior collicular neurons code the timing and the value of the reward in monkeys

Authors: *H. NISHIMARU, Q. V. LE, J. MATSUMOTO, Y. TAKAMURA, E. HORI, T. ONO, H. NISHIJO;
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Abstract: Superior colliculus (SC) plays an important role in guiding the attention to a salient visual stimulus. However, how the SC codes temporal processing of a behaviorally relevant stimulus remains unknown. In the present study, we examined the neuronal activity in the SC while the monkey performed a delayed non-matching to sample (DNMS) task. In this task, the same visual stimulus (sample) was presented 3 times in a row at a fixed interval before a different visual stimulus (target) was shown. The monkey was required to press a button only when the target was presented to receive a reward. Out of 699 neurons recorded, 128 neurons in the deep layer of the SC showed a gradual increase in the activity (climbing activity) during the interval towards the onset of the stimuli. The response magnitude of such climbing activity increased along with the number of trials. Of these 128 SC neurons, 61 were tested with a DNMS task with different reward size and temporal parameters. The response magnitude significantly increased when the reward size was doubled without changing the temporal parameters. By contrast, it significantly decreased when we randomly changed the duration of the interval and the number of times the sample stimuli were shown. Interestingly, when the interval duration of the trial was either increased or decreased, the peak of the climbing responses shifted towards the end of the interval accordingly after several consecutive trials. These results suggest that the activity related to reward anticipation in the SC reflects temporal attentional orienting associated with reward expectation.

Disclosures: **H. Nishimaru:** None. **Q.V. Le:** None. **J. Matsumoto:** None. **Y. Takamura:** None. **E. Hori:** None. **T. Ono:** None. **H. Nishijo:** None.

Poster

234. Reward, Value, and Decisions

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AMED Grant JP18dm0107146
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Title: Trait and state-dependent risk attitude of monkeys measured in a single-option response task

Authors: *A. FUJIMOTO[1,2], T. MINAMIMOTO[1];
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Abstract: Human and animals show diverse preferences to risks ("trait-like" risk attitude) and shift their preference depending on the state or current needs ("state-dependent" risk attitude). For a better understanding of the neural mechanisms underlying risk-sensitive decisions, useful animal models have been needed. Here we examined the risk attitude of three male monkeys in a single-option response task, in which an instrumental lever-release was required to obtain a chance of reward. In each trial, reward condition, either deterministic (100% of 1, 2, 3, 4 drops of juice) or probabilistic (25, 50, 75, 100% of 4-drop juice) was randomly selected and assigned by a unique visual cue, allowing the monkeys to evaluate the forthcoming reward. The subjective value of the reward was inferred from their performance. Model-based analysis incorporating known economic models revealed non-linear probability distortion in monkeys; unlike previous studies, they showed a simple convex or concave probability distortion curve. The risk preference was stable across sessions, suggesting that our observation reflected the trait-like risk attitude of monkeys. Regardless of the baseline risk preference, all monkeys showed an enhancement of risk preference in a session according to the satiation level (i.e., state-dependent risk attitude). Our results suggest that, without choice or cognitive demand, monkeys show naturalistic risk attitude — diverse and flexible like humans. The current approach may provide a useful animal model of risk-sensitive decisions, facilitating the investigation of the neural mechanisms of decision-making under risk.

Disclosures: **A. Fujimoto:** None. **T. Minamimoto:** None.

Poster

234. Reward, Value, and Decisions

Location: Hall A

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Program #/Poster #: 234.03/Q16

Topic: G.02. Motivation

Support: NIH intramural

Title: Accumbal encoding of effortful output

Authors: *B. A. MATIKAINEN-ANKNEY[1], A. V. KRAVITZ[2];
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Abstract: Obesity rivals smoking as the number one cause of preventable death in the US. Physical activity is one of the greatest predictors of overall health, and activity levels are decreased during obesity. Human neuroimaging data suggests that activity in the nucleus accumbens (NAc) is altered in people with obesity, and that this region may be involved in aspects of physical activity including effortful output. Rodent studies suggest the NAc regulates motivated behavior, including persistence. However, the direct role of the NAc in regulating effortful output, and how this may be disrupted during obesity, is not known. To investigate how activity in the accumbens underlies exertion of force, we are training mice to exert varying degrees of force to obtain a palatable reward. Using transgenic mice expressing cre recombinase in D1 or A2A MSNs and fiber photometry to record cre-dependent GCaMP6s signals, we can then analyze cell-specific NAc neural activity time locked to forceful output. Preliminary data suggests NAc neurons may encode effortful output. Going forward we are continuing to investigate this relationship to determine whether increased force output by a mouse correlates with increased NAc activity, and lastly, how it changes over time as mice become obese.

Disclosures: **B.A. Matikainen-Ankney:** None. **A.V. Kravitz:** None.

Poster

234. Reward, Value, and Decisions

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 234.04/Q17

Topic: G.02. Motivation

Title: Neural coding of reward value in richly modulated spike patterns in monkey ventrolateral prefrontal neurons

Authors: *R. FALCONE[1], M. MCDOUGALL[1], D. B. WEINTRAUB[2], T. SETOGAWA[1], B. J. RICHMOND[1];
[1]Lab. of Neuropsychology - Section on Neural Coding an Computation, NIMH, Bethesda, MD;
[2]Surgical Neurol. Br., NINDS, Bethesda, MD

Abstract: Among its functions, lateral prefrontal cortex (lPFC) is involved in assessing rewards. Monkeys with lesions lPFC lose the ability to integrate the reward value information across multiple domains. We recorded neuronal responses from the area 9/46 of ventrolateral prefrontal cortex (vlPFC) of two monkeys while they were performing a task in which in each trial was offered a reward. The reward value, signaled through its association with a visual cue, was constructed by combining one of 3 reward sizes (2, 4 or 6 drops of water) with one of 3 discounting delays (1, 5 or 10s after the choice). The animal could accept or refuse the offer by releasing the bar after the appearance of the go signal. The monkeys modulated their behavior by accepting or refusing the offer according to the value the monkey attributed to the offer; they were increasingly likely to accept offers as the reward became larger and the delay became shorter. We observed that the reward values were well described by a simple reinforcement learning model for the discounted value of the rewards. In the cue period, that is, the period soon after the visual cue was presented to the animal, 68% (118/173; 2-way ANOVA, *p value* <0.05) of the neurons modulated their firing rate according to the reward size and/or delay. We asked whether vlPFC neurons modulated their activity according to the value that the animal assigned to each offer. The estimated discounted values from the reinforcement model from the behavior were used to correlate with the mean firing rate for each offer, for each neuron. We found that 35% (41/118) of the neurons increased or reduced their firing rate linearly in relation to the discounted value measured from the behavior. The other neurons clearly showed modulation according to both reward size and delay, very few neurons were sensitive to only one factor. Inspection of the spike rasters, vlPFC neurons showed a rich repertoire of spike patterns coding for the reward values. Some neurons had a strong pulse after value cue appeared, others showed a strong pause, and still others showed three phase responses (small pulse followed by a pause followed by a strong pulse). Despite these striking patterns of responses, principal component analysis showed that the value-related information was encoded in the spike count. This analysis showed, however, that the period with the strong value related coding was restricted to a window that began and ended during cue's presence before the imperative target (a small yellow or purple spot) appeared.

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Poster

234. Reward, Value, and Decisions

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Topic: G.02. Motivation

Support: NIAAA T32 AA007468
MH R01 48404

Title: Dopamine neuron response to reward presentation in adolescents: Double dissociation of age and learning system

Authors: *A. M. MCCANE[1], M. A. WEGENER[2], B. MOGHADDAM[1];
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Abstract: The dopamine (DA) system plays a central role in reinforcement learning and goal-directed behaviors. The available data and resulting theories on DA neuron responses during reinforcement learning are primarily from adult subjects. Dysfunction of the DA system during development, especially during adolescence, is implicated in psychiatric disorders, including schizophrenia, mood disorders, addiction, and in maladaptive behaviors such as impulsive decision making. Very little is known, however, about similarities or differences in mechanisms that support reinforcement learning in adults vs adolescents. DA neurons are located in the ventral tegmental area (VTA) and the substantia nigra pars compacta (SN).We simultaneously recorded from VTA and SN neurons in adolescent and adult male rats during two fundamental forms of associative learning: classical (Pavlovian) or instrumental (operant) conditioning. Animals were tested in 5-6 consecutive days. Reward in both cases was one sugar pellet. Rate of learning was similar in both age groups. As learning progressed, phasic responses in DA neurons emerged in both the VTA and SN during the conditioned stimulus (CS) or cue, and during reward delivery. The response to the CS in Pavlovian conditioned animals was robust, similar in adolescents and adults, and observed in the VTA and SN. The phasic response to the cue in operant conditioning was smaller but was also similar in both regions. When comparing between learning paradigms, stark age-related differences emerged during reward delivery. Reward delivery in classically conditioned animals had a far larger impact on activating adolescent VTA and SN neurons compared to adults. On the other hand, reward delivery after instrumental response had a significantly larger impact on activating VTA and SN DA neurons in adults compared to adolescents. When comparing between the two age groups, robust differences were observed between the two learning paradigms. Adolescents displayed a smaller response to reward delivery in instrumental compared to operant conditioning, while adults displayed a similar magnitude of response. These data underscore developmental differences in DA-related

value learning and suggest that distinct DA related mechanisms subserve formation of stimulus-reinforcer and action-outcome contingencies in adolescents compared to adults.

Disclosures: A.M. McCane: None. **M.A. Wegener:** None. **B. Moghaddam:** None.

Poster

234. Reward, Value, and Decisions

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 234.06/R1

Topic: G.02. Motivation

Support: NIGMS-NIH Grant P30GM122733

Title: Effects of repeated intermittent episodes of social stress on the acquisition and extinction of a reward-seeking task

Authors: L. SULLIVAN, H. SHAFFER, *A. DEL ARCO;
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Abstract: The repeated and intermittent exposure to social stress promotes cocaine self-administration several weeks after the last stress episode, which suggests long-term behavioral changes associated with the motivation/reward system. The aim of the present study was to investigate whether intermittent social defeat stress alters the acquisition and extinction of a reward-seeking task. Male Long Evans rats were divided in two groups (control, n= 8; stress, n= 8) and exposed to intermittent social defeat stress (or handling) once every three days for ten days (four stress episodes in total). Then, four days after the last stress episode, anxiety behavior was evaluated in the plus maze. After that, thirty days after the last stress episode, rats were trained and tested in a discriminative-stimulus (DS) reward-seeking task. During the DS task rats were required to engage in or withhold responding on trial-by-trial basis depending on a specific stimulus. Briefly, rats were trained to discriminate between a rewarded (fixed light) and a non-rewarded (intermittent light) stimulus that was shown in a specific nose poke (i.e. right or left) to earn a sugar pellet. When the responses to the non-rewarded cue were less than 20%, animals were trained in the extinction protocol. During the extinction protocol responses to both rewarded and non-rewarded cues did not produce any change (no pellet). Both stressed and control animals acquired the DS task and learned to make more responses (nose pokes) to the rewarded cue and less responses to the non-rewarded cue across sessions ($F_{(7,13)}$= 34.37; p< 0.001, ANOVA). There were no differences in the number of nose pokes to both cues between stressed and control animals. Also, during extinction training, both groups of animals learned to inhibit their response to the previously rewarded cue. However, stressed animals made less nose pokes during the first extinction session than controls (stressed= 29 ±2; control= 38 ±2; $F_{(1,13)}$= 7.16; p= 0.019, ANOVA), which indicates a faster extinction learning. In the plus maze, stressed

animals spent more time in the open arms (98 ±11 s) than controls (52 ±11 s) ($t_{(14)}$= 3.1; p= 0.007, t test), which suggests an increased risk-taking behavior. These results further suggest that the intermittent exposure to social stress produces long-term changes in the neurobiological substrates that regulate the extinction of cue-elicited reward-seeking. *Supported by NIGMS-NIH P30GM122733.*

Disclosures: L. Sullivan: None. **H. Shaffer:** None. **A. Del arco:** None.

Poster

234. Reward, Value, and Decisions

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 234.07/R2

Topic: G.02. Motivation

Title: Reward-encoding across the hippocampus - Nucleus accumbens - Ventral pallidum circuit during a Pavlovian conditioning approach task

Authors: *D. SIU[1], A. M. AHRENS[1], M. VALERA[2], V. HETRICK[1], O. J. AHMED[1,2,3,4,5]; [1]Dept. of Psychology, [2]Neurosci. Grad. Program, [3]Dept. of Biomed. Engin., [4]Kresge Hearing Res. Inst., [5]Michigan Ctr. for Integrative Res. in Critical Care, Univ. of Michigan, Ann Arbor, MI

Abstract: The Pavlovian conditioned approach (PCA) task examines how rats interact with a cue (a lever) that is presented for 8 seconds before the delivery of a reward. While there have been electrophysiological recordings performed during the PCA task in the ventral pallidum (VP) and nucleus accumbens (NAcc), there have been no studies recording from the hippocampus. Similarly, to our knowledge, no studies have recorded simultaneously from across all of these structures, making it possible to compare the precise dynamics of these interconnected regions. We have used large-scale tetrode recordings to simultaneously record single units from the dorsal CA1, NAcc core, NAcc shell, and VP regions in male Sprague-Dawley rats. Here, we present the comparative strengths and latencies of cue- and reward-related response dynamics across the hippocampus - nucleus accumbens - ventral pallidum circuit.

Disclosures: D. Siu: None. **A.M. Ahrens:** None. **M. Valera:** None. **V. Hetrick:** None. **O.J. Ahmed:** None.

Poster

234. Reward, Value, and Decisions

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Program #/Poster #: 234.08/R3

Topic: G.02. Motivation

Support: National Institute of Mental Health Intramural Program

Title: Spatiotemporal dynamics of reward processing in basal ganglia and cortex revealed by magnetoencephalography

Authors: L. SEPE-FORREST[1], ***F. W. CARVER**[1], R. QUENTIN[2], T. HOLROYD[1], A. C. NUGENT[1];
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Abstract: Human brain imaging techniques have allowed us to identify individual brain structures involved with the reward system. Nevertheless, the spatiotemporal behavior of these neuronal networks remains largely unexplored. Magnetoencephalography (MEG) allows high temporal and spatial resolution, but its localization of deep structures involved in reward processing has been limited. In this study, we used MEG to analyze reward circuitry during a gambling game. Ten subjects were presented with two cards displaying the numbers 25 and 5 side-by-side. They would choose to bet either 25 or 5 by pressing the left or right button. After their selection, the cards would change colors with green indicating a gain and red indicating a loss of 5 or 25 cents. Some of the cards were randomly 'boost cards' meaning if they were chosen they would turn yellow and indicate a gain of 50 cents. MEG signal was recorded with a 275 channel CTF system and source analysis was conducted using synthetic aperture magnetometry (SAM) beamformers. We compared power in each frequency band across the one-second period after the feedback onset in the boost and the loss of 25 conditions. Significant differences were found in the beta band (13-30 Hz). This is consistent with animal and deep brain stimulation literature which have found changes in beta activity in response to rewarding stimuli or dopamine agonists during in vivo recording. Whole brain activity analyses in the beta band revealed maximal contrast between the boost and loss 25 conditions in the left insula, putamen, dorsal cingulate, and superior temporal gyrus (T-test, $p < .0068$, FDR corrected). Post-hoc analyses of sliding time windows revealed reduced power in the boost condition versus loss-25 condition peaking at 150ms in the insula, 500ms in the dorsal cingulate, and 550ms in the superior temporal gyrus. In addition, there was a prominent increase in beta power in the putamen at 250ms in the boost versus loss-25 condition. In cortical areas, reduced power indicates desynchronization. This task-related beta-band desynchronization signifies an increase in activity and information transfer. However, in the basal ganglia we saw increased power in beta, which indicates a different signature of activity in this region during reward processing

compared to the cortex. These results support and extend existing literature regarding brain networks in reward processing and demonstrate the feasibility of visualizing deep structures using MEG.

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Poster

234. Reward, Value, and Decisions

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 234.09/R4

Topic: G.02. Motivation

Title: Reward processing and functional connectivity in psychiatric patients with substance use problems

Authors: *H. OH[1], S. GOSNELL[2], R. SALAS[1];
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Abstract: INTRODUCTION: Substance use is a major mental health problem characterized by the persistence of drug use despite negative effects. We studied reward processing and resting state functional connectivity (RSFC) between the orbitofrontal cortex (OFC), dorsal striatum, and habenula in psychiatric inpatients with either problem use (PU) or low substance use (LU). METHODS: Psychiatric inpatients (N = 154) were recruited at The Menninger Clinic (Houston TX) and divided into PU and LU based on ASSIST (Alcohol, Smoking and Substance Involvement Screening Test) scores. According to guidelines, subscales for each drug were divided into LU (scores 0-3) and PU (scores 4+) except for alcohol (LU: 0-10 and PU: 11+). Demographic characteristics and psychiatric diagnoses (except for substance or alcohol use disorders) were matched between PU and LU. Participants were scanned in a 3T Siemens Trio MR scanner in the Center for Advanced MRI at Baylor College of Medicine. A 4.5 min MPRAGE (1 mm isotropic voxels, TE = 2.66 ms, TR = 1200 ms) and 5 min resting state scan (3.4x3.4x4 mm voxels, TE = 40 ms, TR = 2s) were collected, followed by 4 functional sessions (3.4x3.4x4 mm isotropic voxels, TE = 40 ms, TR = 2s). The first two sessions included 55 normal events (a 1 s duration yellow light followed by sweet juice 6 s later). The last two sessions included 24 normal events and 12 catch events (juice delivery delayed by 4s). RSFC data and juice reward experiment data were analyzed in CONN toolbox and AFNI, respectively. RESULTS: Preliminary data revealed that the group main effect of juice delivery was observed in dorsal striatum (putamen and caudate), thalamus, insula and inferior frontal gyrus in both PU and LU ($p < 0.0001$, uncorrected). When the PU were compared to the LU, larger responses were found in the left superior frontal gyrus, right inferior frontal gyrus and left caudate ($p < 0.05$, uncorrected). We found higher RSFC between the left medial OFC, the left dorsal striatum, and

the habenula in PU than LU. Additionally, we found that PU showed higher RSFC between the OFC and the insula. CONCLUSIONS: Our findings suggest that RSFC between the OFC and the dorsal striatum, and the habenula and both OFC and dorsal striatum is increased in PU, which implies the habenula may be a part of the same circuit. Moreover, given the results from the preliminary juice experiment data, reward processing in dorsal striatum and superior/inferior frontal gyrus may be altered in PU. Additional research focusing on examining reward processing within these areas could help inform a better understanding of the fronto-striatal and meso-limbic circuits which are involved in substance use.

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Poster

234. Reward, Value, and Decisions

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Topic: G.02. Motivation

Support: Wellcome Trust Sir Henry Dale Fellowship 206207/Z/17/Z
Government Fellowship for Studying Abroad (Cognitive neuroscience: decision behaviour and affective neuroscience), Ministry of Education, Taiwan (2017-2021)

Title: Influences of nutrient rewards on food choice in monkeys

Authors: *F.-Y. HUANG[1], M. SUTCLIFFE[2], F. GRABENHORST[1,3];
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Abstract: The sensory and nutrient properties are intrinsic determinants of a food's reward value. Primates in particular are experts in scrutinizing food objects for their sensory features and nutrient content to make sophisticated decisions. To investigate the effects of nutrient rewards on decision-making, we studied the choice behavior of monkeys for liquid rewards with defined sensory and nutrient components. We study rhesus macaques (*Macaca mulatta)* because their discriminating food choices approximate those of humans; we specifically focus on fat and sugar as these nutrients are effective rewards in humans and clinically relevant to obesity. Three adult male rhesus macaques (V: 11.5 kg; Ya: 17.5 kg; Ym: 11.5 kg) performed a binary choice task in which they repeatedly chose between liquids with different nutrient composition that were offered in varying amounts (0.15 - 0.8 mL). We controlled nutrient composition by using milks with different fat contents to which we added specific sugar amounts and flavors. Liquid type and amount were cued by pre-trained images and magnitude bars, respectively, presented on a

computer monitor. We found that the monkeys were highly motivated by dairy-based rewards and consistently worked for hundreds of trials each testing day. (V: 62,027 trials, 139 sessions; Ya: 25,573 trials, 42 sessions; Ym: 13,933 trials, 28 sessions). Although the monkeys' choices were strongly influenced by offered liquid amounts, nutrient composition had a substantial, additional effect on choice: the monkeys preferred liquids that were high in sugar and fat content. These effects generalized across animals; however, the monkeys also showed subjective, idiosyncratic preferences for different nutrients. Specifically, the monkeys differed in the extent to which they based their choices on nutrient content irrespective of liquid amount, and they valued fat and sugar content differently in making their choices. The influence of fat content on choices could be attributed to a subjective weighting of physical stimulus parameters related to oral texture, implicated in oral fat-sensing. Thus, sugar and fat are potent rewards for rhesus monkeys in controlled choice tasks suitable for neurophysiological recordings. These data indicate that liquid rewards with well-defined nutrient composition can serve as a tool to investigate physical determinants of reward values and their neuronal representations. Understanding the behavioral and neuronal principles underlying nutrient rewards in monkeys has further implications for eating behavior and obesity in humans.

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Poster

234. Reward, Value, and Decisions

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Program #/Poster #: 234.11/R6

Topic: G.02. Motivation

Support: NIH R21-MH113917

Title: Transcranial alternating current stimulation alters reward-dependent corticostriatal interactions

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Abstract: The striatum is a major hub within the reward circuit. Although a host of studies have shown that activation within the striatum is associated with the anticipation of and consumption of reward, recent work has suggested that connectivity with ventrolateral prefrontal cortex (VLPFC) can determine reward-related functions (Smith et al., 2016, Scientific Reports). Yet, it remains unclear whether these corticostriatal interactions play a causal role in the striatal response to reward. To examine this issue, we applied 10 Hz transcranial alternating current stimulation to the VLPFC and a control site (temporal-parietal junction; TPJ) while participants

(N = 32) played a popular card-guessing task used to study reward consumption (Delgado et al., 2000, Journal of Neurophysiology). On each trial, participants were asked to guess whether the number on a mystery card was above or below the number 5. Correct guesses increased the likelihood of winning a monetary bonus while incorrect guesses reduced the likelihood of earning a monetary bonus. Trials were presented in blocks of mostly reward or mostly punishment (i.e., 75%), and each block of trials lasted 30 seconds. Task blocks were separated by fixation blocks lasting 20 seconds. We applied tACS during each task block and recorded neural responses with functional magnetic resonance imaging. Consistent with prior work, receipt of reward (relative to punishment) evoked activation in the ventral striatum. Contrary to our hypothesis, however, tACS applied to VLPFC (relative to TPJ) did not alter striatal responses to reward or punishment. Instead, tACS applied to VLPFC decreased reward-dependent connectivity with the precuneus. Taken together, these results suggest that tACS applied to cortical regions that are connected to the striatum can have downstream effects on reward-dependent corticostriatal interactions. Understanding how tACS can be used to modulate reward-dependent corticostriatal interactions may have important implications for psychopathologies characterized by aberrant corticostriatal responses to reward.

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Poster

234. Reward, Value, and Decisions

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Topic: G.02. Motivation

Support: CONACYT 253631
DGAPA-UNAM IN203518
Technical support: Camacho F.

Title: Comparison of motivated behaviors: Incentive and rewarding values of paced mating and voluntary wheel running

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Abstract: Motivated behaviors such as paced mating (PM) and voluntary wheel running (WR) induce positive affective states in rats as evaluated by the conditioned placed preference (CPP) paradigm. Moreover, it was shown that both activities increase neurogenesis in the olfactory

bulbs and the hippocampus, respectively. The aim of the present study was to compare the incentive and rewarding value of both activities. Ovariectomized female Wistar rats hormonally treated were used. In a first experiment, they were subjected to the following conditions a) WR: 30 min of WR; b) WR then PM: 30 min of WR then 30 min of PM; c) PM then WR: in another group of females the order of behaviors was reversed and consisted in 30 min of PM then 30 min of WR; d) PM + WR: Females had a 1-hour session of WR and PM simultaneously. In a second experiment, the rewarding properties of both activities were evaluated by CPP. Females were assigned to 4 different groups: i) Control; ii) WR: 1 hour of WR; iii) PM: 1 hour of PM; iv) PM + WR: Females had a 1-hour session of WR and PM simultaneously. Physical activity was measured as the time spent running. All parameters of sexual behavior were registered in males: mount, intromission and ejaculation latencies and frequencies, post-ejaculatory interval (PEI) and inter-intromission interval; and females: lordosis quotient and intensity, percentage of exits after mount, intromission or ejaculation and the respective return latencies. The first experiment showed that when females had the choice between PM and WR, they spent less time WR but they executed both activities. Moreover, the time spent on the WR was similar if they had the PM session before or after. These results indicate that both activities have similar incentive values for females. Preliminary results of the second experiment suggest that females from WR and WR + PM group increased their preference index after conditioning, meaning that WR induced a positive affective state.

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Poster

234. Reward, Value, and Decisions

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Topic: G.02. Motivation

Support: NIMH Grant R15-MH110876

Title: Neuronal responses to actions and outcomes in rat ventral pallidum

Authors: E. KRELL, *M. J. FRANCOEUR, K. DEEGAN, G. MACEDONIA, B. M. GIBSON, R. G. MAIR;
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Abstract: Ventral pallidum (VP) is positioned at an intersection of motor- and reward-related circuitry in the brain. In rodents, the activity of VP has been associated with motor behaviors as well as processing of rewarding stimuli, consistent with a role for VP in reward-motivated behavior. Past literature suggests that this role involves the pathway from VP to mediodorsal

thalamic nucleus (MD) and MD to medial prefrontal cortex (mPFC). The present study examines the activity patterns of individual neurons in VP during a reward-guided decision making task. We used single-unit electrophysiology to record VP neurons in Long-Evans rats during performance of a delayed nonmatching to position task. This task requires a decision between two possible lever press responses with positive reinforcement for correct choices. We hypothesized that VP contains neurons that respond selectively to actions (e.g., lever press) and others that respond selectively to outcomes (e.g., positive reinforcement) during the task. Previous research in our lab has found similar cell response types in both MD and mPFC during the same task. After recording, signals from single VP neurons were isolated by spike sorting. The activity of these cells was then analyzed using raster plots and peri-event time histograms (PETH) to identify cells with responses correlated to action and outcome events. In PETH analyses, event-related cells were defined as those with activity above or below the 99% confidence interval (indicating excitation or suppression, respectively) at the time of the event. Raster plots and PETH showed individual neurons with selective responses to relevant events, including excitation at lever presses and excitation or suppression at reinforcement. Encoding of actions and outcomes by VP neurons has potential implications for disorders such as addiction, which involve associations between behavioral decisions and the rewards they elicit. This study suggests a possible contribution of VP to this processing at the cellular level.

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Poster

234. Reward, Value, and Decisions

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 234.14/R9

Topic: H.01. Animal Cognition and Behavior

Support: Whitehall Foundation
Kaufman Foundation

Title: Striatal stimulation reinforces, but does not select, naturalistic untrained behavior

Authors: *A. T. HODGE[1], M. A. NICHOLAS[1], J. T. DUDMAN[2], E. A. YTTRI[1];
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Abstract: Appropriate and effective intentional behavior requires integrating information from previous actions to modify the current action. The basal ganglia are a set of subcortical nuclei composed of two opponent pathways that sit at the intersection of multiple motor circuits and are integral to intentional behavior. Spiny projection neurons (SPNs) in the striatum, input nucleus of the basal ganglia, are organized into two pathways canonically thought to promote (direct

pathway) and inhibit (indirect pathway) movement. Manipulation of the activity of these pathways in the dorsal striatum has also been shown to reinforce specific behaviors and components of behavior (either positively or negatively) based on past experience. Our previous findings suggest that small, short-duration stimulation in the dorsal striatum similar to naturally occurring activity should be able to reinforce any behavior, regardless of action or the presence or absence of an associated goal. Here, we show that unilateral stimulation of SPNs in dorsal striatum of adult, age-matched mice (n=103 sessions, 14 animals, across four groups) is sufficient to reinforce, but not evoke, natural untrained behavior without the presence of an objective, motivating goal. We paired optical stimulation of direct and indirect pathway SPNs with specific untrained behaviors – starting, stopping, ipsilateral turning, and contralateral turning. Stimulation of dSPNs paired with these untrained behaviors resulted in a higher probability of the stimulated behavior. Conversely, iSPN stimulation paired with starting, stopping, and ipsilateral turning resulted in a decreased probability of the stimulated behavior. Unilateral stimulation of dSPNs and iSPNs did not result in any change in the probability of contralateral turn performance. These data demonstrate that the opponent pathways of the basal ganglia act in the reinforcement of specific behaviors, rather than the gating of general movement or the selection of lateralized movement. Further, the history-dependent model of behavioral modulation through reinforcement can be generalized and explain broad variety of motor and cognitive behaviors. This in turn suggests that prolonged stimulation of SPNs generates movement through manipulation of downstream motor nuclei rather than direct alteration of action selection.

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Poster

234. Reward, Value, and Decisions

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Program #/Poster #: 234.15/R10

Topic: H.01. Animal Cognition and Behavior

Support: 5 R01 MH045573-28

Title: Neurocircuitry facilitating coordination between action plan switching and selection regions

Authors: *C. KORPONAY, L. TRAMBAIOLLI, W. TANG, S. HABER;
McLean Hosp., Belmont, MA

Abstract: The ability to flexibly switch action plans in the face of changing outcome values and contingencies is crucial to adaptive functioning. Deficits in action plan switching and selection

are core features of neuropsychiatric conditions such as addiction and obsessive-compulsive disorder. Two spatially distant frontal cortical areas - the anterior cingulate cortex (ACC) and ventrolateral prefrontal cortex (vlPFC) - and the striatum have been centrally implicated in action plan switching and selection. An outstanding question is what anatomical circuitry facilitates coordination between the ACC, vlPFC and striatum to switch between and select action plans, and what specific subregions of these large areas interact with one another. Here, we use anatomical tract-tracing in non-human primates (NHPs) to elucidate 1) the corticocortical connections between ACC and vlPFC subregions and 2) the convergence of these subregions' corticostriatal projection terminal fields in the striatum. First, we find that the strength of corticocortical connections between ACC and vlPFC varies significantly by subregion, and that strongly projecting subregions from one area do not necessarily receive strong projections back from the subregions they innervate in the other area. The most substantial projections from ACC to vlPFC originate in pregenual and postgenual area 24, and project with the greatest strength to vlPFC areas 47, 47L, and 45. On the other hand, the most substantial projections from vlPFC to ACC originate in area 47o, and terminate in areas 32 and 24. Second, we find that all of the ACC-vlPFC subregion pairs that display substantial corticocortical connectivity also display substantial terminal field convergence in the striatum, but in distinct striatal subregions. For instance, while terminal fields from areas 32 and 47o converge in the rostral pole, ventromedial striatum, and along the caudal medial wall, terminal fields from areas 24 and 47L converge in the ventrolateral caudate. Overall, findings suggest that there are specific sets of ACC and vlPFC subregions that interact most strongly with one another, and that coordination between these subregion sets may occur both via direct corticocortical connections and via downstream convergence of corticostriatal projections in distinct zones of the striatum. These findings provide an anatomical foundation for interpreting functional neuroimaging findings on the activity and interactions between the ACC, vlPFC and striatum during action plan switching and selection.

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Poster

234. Reward, Value, and Decisions

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Program #/Poster #: 234.16/R11

Topic: G.02. Motivation

Support: KAKENHI 18K11485

Title: Distinguishing value-coding neurons from random walk neurons in block-structured experiments

Authors: *T. HIWATASHI[1], A. Y. WANG[2], N. UCHIDA[2], K. MIURA[1];
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Abstract: The ability to predict future rewards based on past experiences is essential for animals to select appropriate actions. Neurons in the striatum were reported to code action values, or the expected amounts of reward associated with a specific action, using decision-making paradigms in which reward conditions were altered across blocks of trials (Samejima et al., 2005, Wang et al., 2013). However, a recent study showed that even the simulated "random walk" neurons can be falsely judged to be action value-coding neurons by a conventional test (e.g. t-test) even if they are not correlated with values (Elber-Dorozko et al., 2018). This raises the question of what analysis methods are appropriate to correctly identify value coding neurons. In this study, we sought to explore conditions in which these false detections occur.First, we simulated neural activities using a random walk model, and examined how varying timescales of random walk affect the results. We confirmed that the random walk model can result in false positive value-coding neurons. Interestingly, false-positive identification occurred even if the number of trials is large: the false positive rate ($p<0.05$ or $|t|>1.96$, t-test) did not converge to the chance level (=0.05) but remained at an asymptotic level with increasing trials. To examine if it is caused by the long temporal autocorrelations of random walks, we considered the refined (autoregressive) model, whose timescales of autocorrelations are very short. Surprisingly, the simulated neural activities were still misjudged as value-coding neurons even with a large number of trials.We next explored the mathematical basis of why false-positive identification can occur. Although the action values (=block structure) and random walks are not correlated on average, they can be incidentally correlated on a session basis. For example, a simulated neuron's activity can be positively correlated with action values in one simulation session ($t>0$), while it can be negatively correlated in another ($t<0$). Our analysis showed that the key factor is not the mean (=0) but the variance of the t-value distributions, which turned out to be proportional to the block size (i.e. the number of trials in which the reward amounts are fixed). And, theoretically, false-positive identification occurs minimally at the chance level in the short block size limit, i.e. when the block size is one.In conclusion, the autocorrelations of neural activities, however short, as well as block experimental structures leads to the false detection of value-coding neurons in the conventional t-test. These results provide insights into how to develop more appropriate statistical test in the future.

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Poster

234. Reward, Value, and Decisions

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Title: Nucleus accumbens D1- and D2-MSNs drive both reward and aversion

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Abstract: One of the key regions of the reward circuit is the nucleus accumbens (NAc), which is mainly composed by D1-MSNs, classically associated with reward, and D2-MSNs, associated with aversion. However, recent work has challenged this functional dichotomy, suggesting a much more complex system than initially anticipated.In our work, we show that that both MSN subpopulations can drive reward and aversion, depending on their neuronal stimulation pattern. Brief D1- or D2-MSN optogenetic stimulation elicited positive reinforcement and enhanced cocaine conditioning. Conversely, prolonged activation induced aversion, and in the case of D2-MSNs, decreased cocaine conditioning. Brief stimulation was associated with increased VTA dopaminergic tone either directly (for D1-MSNs) or indirectly via ventral pallidum (VP) (for D1- and D2-MSNs). Importantly, prolonged stimulation of either MSN subpopulation induced remarkably distinct electrophysiological effects in these target regions. We further show that the effects of prolonged stimulation are dependent on the activation of distinct opioid receptors in the VTA and VP. Our findings demonstrate that D1- and D2-MSNs can bi-directionally drive reward and aversion, explaining the existence of controversial studies in the field, and highlights that additional studies need to be performed to further understand the role of these subpopulations in behavior.

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Poster

234. Reward, Value, and Decisions

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 234.18/R13

Topic: G.02. Motivation

Support: JSPS KAKENHI Grant Number JP16H06567

Title: Striatal neurons monitor dynamically changing reward value by their sustained activity in monkeys

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Abstract: In our daily life, the value of rewards dynamically changes even by the second. For instance, when you pour a beer into your beer mug, the amount (i.e., value) of the beer that you will obtain increases by the second. How does the brain monitor such dynamically changing reward value? Although many cortical and subcortical structures have been shown to encode reward value under an ideal condition in which the reward value does not change with time, it is unclear whether and how these structures monitor dynamically changing reward value. To address this issue, we recorded the activity of presumed projection neurons from the striatum, which are known to encode reward value in the ideal condition, in monkeys during a classical conditioning. In this conditioning, a bar stimulus was presented as a conditioned stimulus (CS) and the length of the bar indicated the amount of a liquid reward (US) that the monkey would obtain. The bar length changed by the second in three different ways. In the first condition (value-increase condition), the bar length gradually increased, and the gradual increase randomly stopped within 2450 ms. In the second condition (value-decrease condition), the bar length gradually decreased, and the gradual decrease randomly stopped within 2450 ms. In the third condition (value-fixed condition), the bar length did not change and was fixed at a short length (i.e., small reward, 0.1 ml), medium length (medium reward, 0.2 ml) or long length (large reward, 0.3 ml). We found that many striatal neurons changed their sustained activity as the bar length changed with time. Of 258 recorded neurons, 35 neurons exhibited a significant, gradual increase in their activity as the bar length (i.e., value) increased with time in the value-increase condition ($P < 0.05$). In the fixed-value condition, these neurons were more strongly activated when a longer bar was presented (i.e., when a larger reward was predicted). On the other hand, 46 of the 258 neurons exhibited a significant, gradual increase in their activity as the bar length (i.e., value) decreased with time in the value-decrease condition ($P < 0.05$). In the fixed-value condition, these neurons tended to be more strongly activated when a shorter bar was presented (i.e., when a smaller reward was predicted). These activity patterns suggest that the 35 and 46

neurons encoded both the fixed value and the gradually-changing value in a consistent manner in which their activities were positively and negatively correlated, respectively, with the values. Thus, striatal neurons monitored dynamically changing reward value by their sustained activity.

Disclosures: O. Toyoshima: None. **Y. Wang:** None. **H. Yamada:** None. **M. Matsumoto:** None.

Poster

234. Reward, Value, and Decisions

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Program #/Poster #: 234.19/R14

Topic: G.02. Motivation

Support: JSPS KAKENHI Grant Number JP16H06567

Title: Tonic firing of midbrain dopamine neurons monitors dynamically changing reward value in monkeys

Authors: *Y. WANG[1], O. TOYOSHIMA[1], J. KUNIMATSU[1,2], H. YAMADA[1,2], M. MATSUMOTO[1,2];
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Abstract: Midbrain dopamine neurons have been shown to encode the value of rewards by their "phasic" response. These experiments were conducted under an ideal condition in which the reward value was temporally stable and did not change with time, although we often experience that the reward value dynamically changes even by the second. For instance, when grilling a steak, its palatability (i.e., its value) changes by the second. How do dopamine neurons monitor such dynamically changing reward value? In the present study, we found that dopamine neurons changed their "tonic" firing as an expected reward value changed by the second. We recorded single-unit activity from dopamine neurons in monkeys during a classical conditioning. In this conditioning, a bar stimulus was presented as a conditioned stimulus (CS) and the length of the bar indicated the amount of a liquid reward (US) that the monkey would obtain. The bar length changed by the second in three different ways. In the first condition (value-increase condition), the bar length gradually increased, and the gradual increase randomly stopped within 2,450ms. In the second condition (value-decrease condition), the bar length gradually decreased, and the gradual decrease randomly stopped within 2,450ms. In the third condition (value-fixed condition), the bar length did not change and was fixed at a short length (i.e., small reward, 0.1 ml), medium length (medium reward, 0.2 ml) or long length (large reward, 0.3 ml). Of 68 recorded dopamine neurons, 13 neurons exhibited a significant, gradual increase in their tonic activity as the bar length (i.e., value) increased with time in the value-increase condition while only 2 neurons exhibited a significant, gradual decrease ($p < 0.05$). We confirmed that these

neurons encoded the fixed value by their phasic response in the fixed-value condition. On the other hand, 7 of the 68 recorded neurons exhibited a significant, gradual decrease in their tonic activity as the bar length (i.e., value) decreased with time in the value-decrease condition while only 2 neurons exhibited a significant, gradual increase ($p < 0.05$). These neurons also encoded the fixed value by their phasic response in the fixed-value condition. Our findings suggest that a subset of midbrain dopamine neurons monitors reward value not only by their phasic response but also by a tonic, gradual change in their activity when the value is changing by the second.

Disclosures: Y. Wang: None. **O. Toyoshima:** None. **J. Kunimatsu:** None. **H. Yamada:** None. **M. Matsumoto:** None.

Poster

234. Reward, Value, and Decisions

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Program #/Poster #: 234.20/R15

Topic: G.02. Motivation

Support: R01DA042038
R01NS104834
MQ

Title: Serotonin neurons drive metalearning during dynamic foraging

Authors: *C. D. GROSSMAN[1], B. A. BARI[1], A. S. GINSBERG[2], E. E. LUBIN[2], J. Y. COHEN[1];
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Abstract: In order to survive, animals must navigate complex and dynamic environments. Doing so requires using previous experience to guide behavior and updating behavior with each subsequent experience. These processes should not be rigid; rather, they should adapt to match the statistics of the environment. The neural mechanisms of learning and decision making that underlie this flexible behavior, and how they are adjusted to maximize success, are largely unknown. Prior research points to a role for neuromodulatory neurons in these processes. In particular, serotonin neurons in the dorsal raphe are hypothesized to regulate flexible decision making after a change in the contingency between an action and the outcome that it produces. To test this hypothesis, we designed a dynamic foraging task for head-fixed mice that is amenable to extracellular electrophysiological recordings from optogenetically-identified serotonin neurons. Mice exhibited flexible behavior in this probabilistic and dynamic environment, using reward history to select actions and successfully harvest rewards. We found correlations between serotonin neuron activity and recent reward history, regardless of the actions taken to acquire

those rewards. Lesions of serotonin neurons resulted in perseverative behavior, i.e., increased the likelihood of making the same decision as on the previous trial, no matter what the outcome. To understand these findings, we adapted a metalearning reinforcement learning model that uses reward history to guide decision making. Intuitively, average reward rate may be useful as feedback about of the success of a behavioral policy that can be used to make changes to that policy. In this model, reward rate is used to modulate the tendency to exploit the action with the highest expected value or explore other available actions. Using a hierarchical Bayesian approach to model fitting, we found that the activity of serotonin neurons correlated with the model-generated average reward rate. Further, we show that the lesion effectively removed this dynamic, reward-history-dependent modulation of exploration and exploitation. Taken together, these findings suggest that dorsal raphe serotonin neurons integrate overall reward history to modulate flexible decision making in a manner consistent with the proposed metalearning model.

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Poster

234. Reward, Value, and Decisions

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Program #/Poster #: 234.21/R16

Topic: G.02. Motivation

Support: R01DA042038
R01NS104834
MQ

Title: Subthreshold basis for bidirectional persistent activity in neocortex

Authors: *E. KIM, B. A. BARI, J. Y. COHEN;
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Abstract: Nervous systems are capable of maintaining information internally. This information is then used in complex cognitive behaviors such as working memory, attention, biasing decisions, and predicting future events. Neurons in neocortex show persistent activity changes, which are thought to represent this internal process. The mechanisms by which this activity arises are varied and incompletely understood. Here, we studied prefrontal cortex (PFC) in mice performing a behavioral task in which different stimuli predicted rewards at different delays. We measured the membrane potential (*Vm*) and action potentials from pyramidal neurons across layers in PFC. We found that the majority of PFC neurons showed bidirectional persistent firing rate changes that arose due to sustained changes in mean and variability of *Vm* during the delay. Persistent maintenance of *Vm* changes was robust to perturbation, indicating it was stable.

During reward trials this activity was terminated by an external stimulus (reward delivery). Interestingly, no-reward trials also showed persistent *Vm* changes which terminated at the precise time of the longest possible reward delay, without any external stimuli. This indicates that persistent activity can represent a purely internal state. Persistent activity was layer (L) specific: upper L5 neurons showed persistent depolarization in the delays to reward, lower L5 neurons showed persistent hyperpolarization, and L2/3 neurons did not show persistent activity. Neurons in sublayers of L5 showed differences in intrinsic properties indicating two distinct populations of L5 neurons showing bidirectional persistent activities. These findings reveal that reward-predictive persistent activity in PFC is temporally and spatially organized, and also conveys information about internal state via synaptic mechanisms.

Disclosures: E. Kim: None. **B.A. Bari:** None. **J.Y. Cohen:** None.

Poster

234. Reward, Value, and Decisions

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Program #/Poster #: 234.22/R17

Topic: G.03. Emotion

Support: NRF - 2016M3C7A1914448
NRF - 2017M3C7A1031331

Title: Somatic marker influences a decision making under uncertainty

Authors: *J. KIM[1], B. JEONG[2];
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Abstract: The somatic marker hypothesis proposed that an emotive cue or event evokes an interoceptive processing called ‘somatic marker’ that influence the decision making. Furthermore, this somatic marker has been known to be involved in the subjective emotional feeling. However, it has rarely been investigated whether cortical interoceptive processing (somatic marker) changes during the decision making to influence a decision and its relationship with subjective feeling. In our previous study, we have shown that change in the aversive emotional context (expecting blame) induces a change of decision tendency during a decision making under uncertainty. We hypothesized that cortical interoceptive processing would be modulated by an emotional context and would influence subsequent decision under uncertainty. In this EEG study, using the heartbeat-evoked response (HER) as an index of cortical interoceptive processing, and computational modeling of the behavior, we have shown that cortical interoceptive processing changes as the probability of the emotional blame increases, suggesting the existence of the somatic marker. Furthermore, a change of this somatic marker

was associated with irreducible-uncertainty induced suboptimal choices showing evidence of somatic marker-influenced decision making under uncertainty. Finally, this somatic marker was not involved in the processing of danger feeling. These results provide novel evidence of somatic marker by showing modulation of cortical interoceptive processing and its influence of uncertain decision.

Disclosures: J. Kim: None. **B. Jeong:** None.

Poster

234. Reward, Value, and Decisions

Location: Hall A

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Topic: G.03. Emotion

Support: DARPA W911NF-14-2-0045

Title: Decoding of cognitive flexibility state across multiple days using temporal and pre-frontal cortical local field potential during the performance of a multi-source interference task

Authors: J. MIRSKY[1], ***I. BASU**[1], A. YOUSEFI[1], Y. AMIDI[1], A. C. PAULK[1], R. ZELMANN[1], G. BELOK[1], K. FARNES[1], D. D. DOUGHERTY[1], Z. WILLIAMS[1], S. S. CASH[1], U. EDEN[2], A. S. WIDGE[3];
[1]Massachusetts Gen. Hosp., Boston, MA; [2]Mathematics and Statistics, Boston Univ., Boston, MA; [3]Psychiatry, Univ. of Minnesota, Minneapolis, MN

Abstract: A rarely covered topic in neuroscience involves identifying underlying cognitive processes, such as cognitive flexibility, with tasks performed across multiple days. These processes can be represented as hidden states estimated using concatenated brain activity and/or behavioral responses across days. In this work, we used a state space framework to estimate a hidden cognitive flexibility state using multiple days' worth of behavior and neural data. Human participants consisted of ten patients with long-standing pharmaco-resistant complex partial seizures who voluntarily participated after fully informed consent. Participants performed a multi-source interference task (MSIT) across 2, 3, and 4 day recording sessions with simultaneous recordings of reaction time (RT) and local field potential (LFP) from cortical and subcortical brain structures. We used a state space modeling framework to first estimate a hidden baseline cognitive flexibility state from RT. We then estimated an encoder model relating the cognitive state to neural features extracted from cortical and subcortical LFPs. These features consisted of spectral power of the LFP in theta (4-8 Hz), alpha (8-15 Hz), 15-30 Hz, 30-55 Hz, 65-110 Hz, and 130-200 Hz bands over a time interval of 2 seconds aligned with image onset. Finally, using the encoder model, we determined a neural decoder to predict the cognitive flexibility state from a subset of neural features. We found that we could use 1-3 neural features

to reliably decode the cognitive flexibility state across 3 and 4 day recording sessions. These features consistently originated from dlPFC and Temporal brain regions at frequency bands 4-8 Hz, 8-15 Hz, and 65-110 Hz.
We found that these features were selected by the encoder for each individual day of RT and LFP data, as well as for all days of data concatenated. In utilizing these features as global variables for decoding, we found a root mean squared error to be less than half of the original value compared to decoding without utilizing these features as global variables. This framework can be used to design closed loop electrical stimulation to improve day-to-day cognitive flexibility in patients with mood and anxiety disorders.

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Poster

234. Reward, Value, and Decisions

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Topic: G.03. Emotion

Support: MGH-MIT Grand and Challenges program
Brain & Behavior Research Foundation
the One Mind Institute
Harvard Brain Initiative Bipolar Disorder Fund supported by Kent & Liz Dauten
NIH
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Title: Assessing the effects of deep brain stimulation of the mid and ventral striatum in cognitive flexibility

Authors: *A. E. REIMER[1], M.-C. LO[2], A. R. DE OLIVEIRA[3], G. J. SIMANDL[1], A. S. WIDGE[2];
[2]Psychiatry, [1]Univ. of Minnesota, Minneapolis, MN; [3]Dept. de Psicologia, Univ. Federal de Sao Carlos, Sao Carlos, Brazil

Abstract: Impaired cognitive flexibility is found in several psychiatric disorders, such as obsessive-compulsive disorder (OCD) and depression. Dysregulation of the striatum and altered corticostriatal connectivity seem to underlie pathological inflexibility. Deep brain stimulation (DBS) of the striatum and internal capsule seems to influence corticostriatal circuitry, possibly ameliorating inflexibility. We assessed the effects of DBS applied to the mid or ventral striatum (mS-DBS and vS-DBS, respectively) in Long Evans rats performing an operant set-shifting

paradigm. DBS was delivered 1 h prior to and during the set-shifting test. Rats performed 15-18 sessions and DBS (130 Hz, 0.1 ms pulse width, 100-300 μA) was administered on alternating days. In a second cohort, we evaluated the effects of vS-DBS in an animal model of inflexibility, meta-Chlorophenylpiperazine administration (5-HT2A/C-agonist, mCPP). The animals received mCPP only (0.5 or 2.0 mg/kg), DBS-only (100 μA), or a combination of drug and DBS. vS-DBS reduced reaction time, without affecting accuracy (RT, $t = 2.77$, $p = 0.015$, for regression coefficient; errors, $t = 0.28$, $p = 0.96$, trials = 16465, n = 13); vVS-DBS had no effect. Additionally, a higher c-Fos expression in the PFC correlated with faster RT mean responses. On the second cohort, mCPP impaired animals' flexibility (RT, $t = -6.30$, $p < 0.01$, trials = 8538, n = 12), but DBS did not reverse mCPP-induced inflexibility (RT, $t = -1.47$, $p = 0.76$). DBS improved flexibility, particularly when applied to the mid striatum. This effect seems to depend on the influence of DBS on frontal areas, such as prelimbic, infralimbic, and orbitofrontal cortex. mCPP's effect on flexibility appears to occur outside corticostriatal circuits. Continued investigation will help to define corticostriatal circuits' influence and DBS' potential benefits on flexibility.

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Poster

234. Reward, Value, and Decisions

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Topic: G.03. Emotion

Support: NIH Grant 1R21MH109722-01A1
NIH Grant 1R21MH113103-01A1

Title: Open source software tools to measure and control oscillatory synchrony of brain regions

Authors: *M. J. SCHATZA, E. B. BLACKWOOD, M.-C. LO, A. S. WIDGE;
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Abstract: To successfully perform their computational functions, brain regions must become functionally connected. This functional connectivity is correlated with oscillatory synchrony, particularly in lower frequency bands. Synchrony can be quantified by the inter-region coherence of local field potential (LFP) recordings. If changes to synchrony cause lasting functional changes, then synchrony modulation would be an approach to treating circuit dysfunctions. However, causal testing of neural synchrony is difficult and unreliable with current tools. Here, we describe open-source software tools that can perform two protocols to monitor and alter synchrony. The first protocol is designed to artificially increase or decrease the oscillatory

synchrony between two brain regions using closed-loop phase-locked stimulation. We have created a real-time phase estimation algorithm to analyze LFP at a “recording” brain region. The algorithm bandpass filters its input to the desired frequency band, predicts forward using an autoregressive model to offset filter delay, and outputs an imaginary component using a Hilbert transformer. The complex angle of the filtered input plus the derived imaginary component defines the phase. When the phase crosses a target threshold, we trigger a pulse in the “controlled” region. To improve accuracy, the threshold can be trained online based on errors from the target at proposed pulse times. The optimized system delivers pulses centered within 5° of any phase target, with a circular standard deviation of &lt80° over the course of an experiment. The second protocol validates the effects of closed-loop stimulation by estimating coherence spectra between pairs of recording channels in real time. The implementation calculates cross- and auto-spectra at frequencies and times of interest during 8-second time windows using frequency-domain convolution with a windowed sinusoid. A running average over many windows is used to obtain magnitude-squared coherence. We will present quantitative comparisons demonstrating equivalence to parallel offline analyses. This protocol allows strategically choosing testing parameters, for example increasing or decreasing synchrony at frequencies with naturally high coherence. Real-time coherence visualization also allows the operator of a closed-loop experiment to ensure that the manipulation is effective. These open-source tools allow neuroscientists to build experiments that directly and causally probe the role of oscillatory coherence in organizing functional brain networks.

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Poster

234. Reward, Value, and Decisions

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Program #/Poster #: 234.26/S1

Topic: G.03. Emotion

Support: São Paulo Research Foundation (FAPESP, 2017/22473-9)
OneMind Institute
the MnDRIVE Brain Conditions Initiative
UMN Medical Discovery Team on Addictions

Title: No effect of repeated orbitofrontal-striatal optogenetic stimulation on repetitive behaviors and behavioral flexibility in rats

Authors: *A. R. DE OLIVEIRA[1,2], A. E. REIMER[2], G. SIMANDL[2], A. S. WIDGE[2]; [1]Psychology, Federal Univ. of Sao Carlos (UFSCar), Sao Carlos, Brazil; [2]Psychiatry, Univ. of Minnesota, Minneapolis, MN

Abstract: Accumulating evidence points to orbitofrontal cortex-ventromedial striatum (OFC-VMS) circuitry hyperactivity as a cause of Obsessive-Compulsive Disorder (OCD). Hyper-activating this pathway in inbred mice produces excessive grooming that outlast the stimulation. We aim to replicate these findings in outbred rats, where there are few reliable models of OCD-related behavior. Optical-fibers were implanted into the VMS and opsin delivered into the OFC (AAV5-CaMKIIa-hChR2(H134R)-EYFP, n=9; AAV5-CaMKIIa-EYFP control, n=8) of male Long-Evans rats. After waiting 5-6 weeks for viral expression, rats received repeated optical stimulation (5 min/day, 6 consecutive days, 10 ms, 10 Hz, 5 mW). Self-grooming was evaluated for 5-min periods: before, during, immediately after and one-hour after each stimulation and also one and two weeks after the ending of the 6-day stimulation protocol. Marble burying, nestlet shredding test, and operant attentional set-shifting sessions were performed before and after the repeated opto-stimulation. OFC-VMS repeated stimulation did not increase grooming immediately before (2-way RM-ANOVA, $F(1,75)=0.70$, $p>0.05$), during ($F(1,75)=0.33$, $p>0.05$), immediately after ($F(1,75)=0.30$, $p>0.05$) or one-hour after stimulation ($F(1,90)=2.37$, $p>0.05$). A small increase in grooming was observed one week after the stimulation protocol ended, but this did not persist to two weeks ($F(1,15)=4.56$, $p=0.05$). No changes were observed in nestlet shredding ($F(1,60)=3.40$, $p>0.05$), marble burying ($F(1,60)=0.50$, $p>0.05$), or set-shifting (Reaction_Time: $t=-0.21$, $p>0.05$; Errors: $t=0.57$, $p>0.05$; for regression coefficient, trials=29740). Stimulation did not affect locomotor behavior ($F(1,75)=2.22$, $p>0.05$). In sum, we could not reproduce in rats a model of compulsive behavior that has previously been reported in mice. The effect in rats appears to be more difficult to create and less penetrant. If optogenetic effects on behavior do not reliably transfer even between rodent species, this may have important implications for designing rodent-to-human translational pipelines.

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Poster

234. Reward, Value, and Decisions

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 234.27/S2

Topic: H.01. Animal Cognition and Behavior

Support: GRACA (Graduate Research and Creative Activity) grant 1549

Title: The effects of latent infection of *toxoplasma gondii* on the domestic cat

Authors: *M. ALYETAMA[1], A. HOFFMANN[2], S. WOMACK[1], G. EVAH-NZOUGHE[2], K. HIGGINS[3], J. STECKELBERG[4], B. A. CHASE[3];
[1]Psychology, [2]Neurosci., [3]Biol., Univ. of Nebraska at Omaha, Omaha, NE; [4]Microbiology, Iowa State Univ., Ames, IA

Abstract: Domestic cats have a worldwide *T. gondii* seroprevalence of 30-40% and play pivotal roles in this parasite's transmission: it reproduces sexually only in felid intestines, after which it is shed in their feces. Asymptomatic *T. gondii* infection is associated with bradyzoite-containing brain cysts that produce tyrosine hydroxylase. Elevated dopaminergic tone is thought to underlie increased locomotor, risk-averse, and exploratory behavior in many infected animals. However, the effect on cat behavior is unknown. We propose that latent *T. gondii* infection in domestic cats similarly alters their behavior to increase its spread. This follows from the well-supported 'fecal-exposure' hypothesis: if *T. gondii* mediates the size of a cat's home range characteristics, then it will direct where soil is contaminated with *T. gondii* and influence parasite exposure in intermediate hosts and in cats with overlapping home ranges. Since many species have subtle behavior alterations following *T. gondii* infection, we are testing whether *T. gondii* alters behavioral characteristics of its definitive host. Specifically, we hypothesize that chronic *T. gondii* infection in domestic cats decreases their risk-averse behavior and leads to increased locomotor activity. We have built an on-line survey (toxoproject.com/survey) to assess understanding of *T. gondii* infection and risks and recruited owners of pet cats from the participants. We then cross-sectionally evaluated whether risk-aversion and locomotory activity in their pets differ by seropositive status. In one behavioral paradigm, cat behavior is recorded over a series of episodes with and without the owner. When the owner is present, s/he sits and interacts with their cat. After an initial set of familiarization episodes, a balanced scent-based paradigm is used to evaluate a risk-averse response. We demonstrate that this least-invasive paradigm is useful to infer behavioral changes that may result from alteration of dopamine tone via *T. gondii* infection. This work provides an understanding of how *T. gondii* infection impacts cat behaviors to influence parasite spread. It also contributes to a theoretical framework to understand the impact of the risk of infection by fecal-transmitted parasites in an ecologically relevant setting.

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Poster

235. Emotion: Positive and Negative Emotional States

Location: Hall A

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Program #/Poster #: 235.01/S3

Topic: G.03. Emotion

Support: MSCA-IF-EF-ST Grant 795994

Title: Stimulus intensity affects emotion regulation success and neural responses in subcortical and cortical regions

Authors: *C. MORAWETZ[1], S. BERBOTH[2], C. WINDISCHBERGER[1];
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Abstract: Introduction Individuals differ widely in their experience of emotions and the ability to regulate them. These individual differences might vary as a function of perceived stimulus intensity. Yet, few studies to date have examined how variability in stimulus intensity (SI) impacts emotion regulation (ER) ability and the underlying neural networks. To address this issue, we used a standard fMRI ER task and parametric analyses on a trial-by-trial basis. **Methods** 20 participants (16f, age: *M*=22.8±3.3yrs) performed a well-established ER task (see Fig. 1A) during three scanning sessions separated by one week. We acquired four runs/session and 80 trials/session using the CMRR multiband EPI sequence (TR=1.4s; TE=23ms; 78 slices; voxel size=1.5x1.5x1.2mm^3) at ultra-high field (7T). 240 aversive images were rated on SI (1 calm to 9 exciting) covering a wide range of arousal (low: 1.69-4.94, M=3.82±0.79; high: 5.06-8.19, M=6.20±0.83). Parametric analyses using emotional state rating (ESR) and SI were performed on the whole sample and a subsample of 14 participants (11f, age: M=22.9±3.8yrs), respectively. **Results** Participants felt significantly less negative during Decrease compared to Look ($t(19)=6.37$, $p<.001$) and ER success was higher for low compared to high arousing images ($t(13)=-5.76$, $p<0.001$). Parametric analysis revealed that responses in the amygdala were positively associated with SI and negatively with ESR independent of the ER condition (Fig. 1B & 1C). Activity in right IPL and dACC was positively correlated with SI and negatively with ESR during Look. This suggests that when participants successfully resist to regulate their emotions, regions implicated in conflict monitoring demonstrate enhanced activity with increasing arousal. Activity in the right DLPFC was negatively modulated by ESR during Decrease, indicating that this region is only affected by ER success. **Conclusion** These findings indicate that SI affects ER ability and predicts neural responses in regions important for encoding the arousal value of a stimulus as well as conflict monitoring and attention.

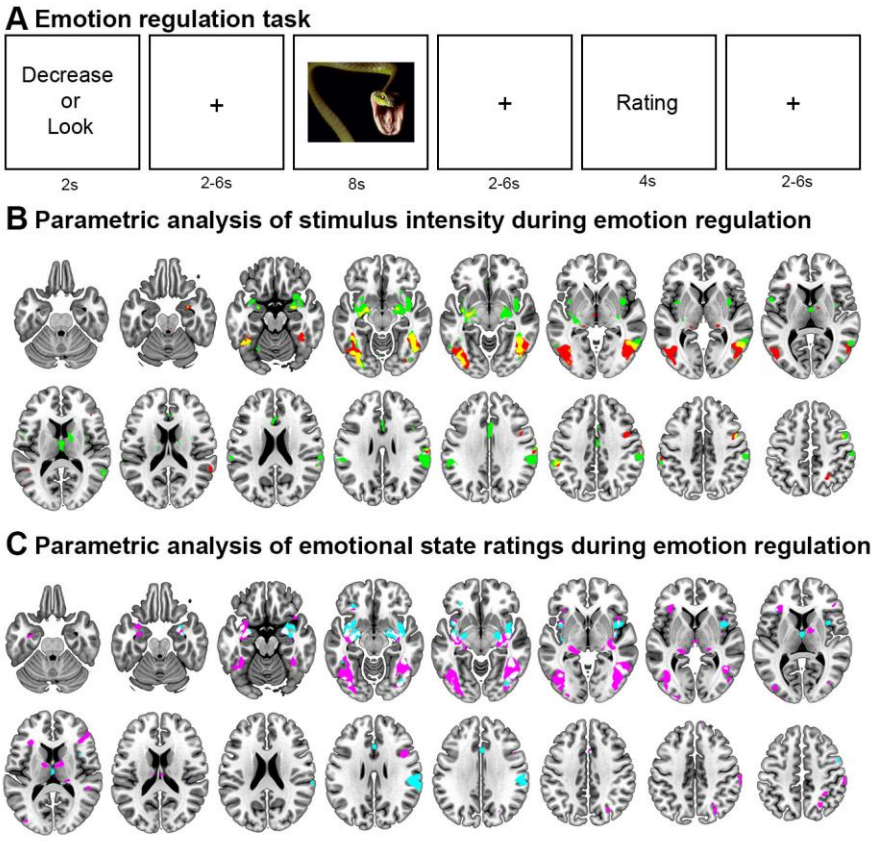

Figure 1. (**A) Task Design.** Participants are instructed to either down-regulate their emotions (Decrease) or attend to an aversive image without modulating their emotions (Look). An event-related design is used. After the instruction (2s), a highly aversive image is presented (8s) and participants decrease their emotions or look at the image. This is followed by a rating (4s), in which subjects indicate their current emotional state on a continous scale from very negative to very positive.
Participants performed the ERT during 3 scanning sessions seperated by 1 week. Each session consisted of 4 runs and a total of 80 trials.
(B & C) Parametric analysis (FWE $p<0.05$). Parametric analysis of trial-by-trial variability in stimulus intensity **(B)** and emotional state ratings **(C)** during the regulation phase. Overlapping activation (**B**, indicated in yellow) in bilateral amygdala and fusiform gyrus scaled positively with stimulus intensity during Decrease (red) and Look (green), while activation in the dorsal anterior cingulate cortex (dACC) and right inferior parietal lobe (IPL) was selectively positively correlated with stimulus intensity during Look. Overlapping activation (**C**, indicated in white) in bilateral amygdala and fusiform gyrus scaled negatively with emotional state ratings during Decrease (purple) and Look (cyan). Ratings were negatively correlated with activation in the right IPL and dACC during Look, and in the left anterior insula and right dorsolateral prefrontal cortex (DLPFC) during Decrease.

Disclosures: C. Morawetz: None. **S. Berboth:** None. **C. Windischberger:** None.

Poster

235. Emotion: Positive and Negative Emotional States

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 235.02/S4

Topic: G.03. Emotion

Title: Investigating the dynamic changes in functional brain networks during emotion regulation using fMRI-informed EEG source localization

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Abstract: Emotion regulation plays a crucial role in daily life, but can be disrupted by disease. To efficiently process external information and regulate emotions, functional brain networks need to rapidly and dynamically reorganize. Unfortunately, the spatiotemporal dynamics of large scale networks are difficult to investigate due to range and speed of cortical function. This study captured this highly dynamic network by performing a sliding time window correlation analysis based on a developed high spatiotemporal resolution fMRI-informed EEG source model. In this experiment, 6 healthy subjects were recruited to perform an emotion regulation task. Subjects were shown randomized neutral or negative stimulus images, and asked to either passively view the image or reinterpret the image in a more favorable way. FMRI and EEG data were concurrently collected during the experiment using 3T MRI scanner and a 64-channel MRI-compatible EEG cap. EEG data were filtered from 0.1 Hz to 40 Hz and segmented from 200ms prior to image onset to 1000ms after onset. FMRI data underwent a conventional preprocessing and GLM-based analysis, and the activation maps were applied to constrain EEG source localization and achieve a high spatiotemporal resolution. Sliding time window correlation analysis over the 1000ms after stimulus onset, using the 200ms before onset as a baseline, and k-means clustering was applied to the windowed correlation matrices strategies to isolate the transient states of functional connectivity. Betweenness centrality was then used to characterize the central hubs of each network. Clustering of the windowed correlation matrices identified four different functional brain states that arose during the process of emotion regulation, with each reflecting a specific functional role. A “top-down” processing mode was observed, with the dorsolateral prefrontal cortex (DLPFC) appearing as a central hub for the generation and reappraisal of emotion. Other brain regions that exhibited brain-state specific peaks in network centrality included the visual cortex, fusiform gyrus, superior temporal cortex, insular, anterior cingulate cortex and posterior parietal cortex. The functional brain networks that arise during emotion regulation are highly dynamic. The DLPFC plays a consistent, central role during this process, and variably coordinates with other regions of the salience, emotion, and visual networks in the generation and regulation of emotion.

Disclosures: **F. Fang:** None. **T. Potter:** None. **R. Li:** None. **T. Nguyen:** None. **Y. Zhang:** None.

Poster

235. Emotion: Positive and Negative Emotional States

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 235.03/S5

Topic: G.03. Emotion

Title: Effects of written emotional expression on health outcomes and salivary cortisol

Authors: *M. E. KNEAVEL[1], E. LOSCALZO[2];
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Abstract: Written emotional expression or journaling about traumatic events has shown positive health benefits in a number of settings (Symth, 1998; Pennebaker & Beall, 1986; Travagin, Margola, and Revenson, 2015) This research was designed to investigate whether this technique could reduce adverse health outcomes in both handwritten and typed formats and if there were effects on cortisol levels. Twenty-four participants were randomly assigned to one of two writing mediums (handwriting or typing) and one of two disclosure protocols (trauma versus control). Health outcomes and salivary cortisol levels were measured at baseline, at completion of the 3-day writing, and at a 3 month follow up. Consistent with previous research, results indicated a significant change in the number of days missed from school or work when writing about a traumatic experience. Journaling about a traumatic experience can be an effective intervention to improved health outcomes and was associated with a reduction in number of days missed; however, unexpectedly there were no significant change in the number of colds, health center visits, or cortisol levels at the three-month follow-up. Results are discussed in terms of the similarities and effectiveness of the typing technique as a useful mechanism for affecting change.

Disclosures: M.E. Kneavel: 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.; National Collegiate Athletic Association - U. S. Department of Defense Mind Matters Challenge. **E. Loscalzo:** None.

Poster

235. Emotion: Positive and Negative Emotional States

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 235.04/S6

Topic: G.03. Emotion

Title: A lack of the negativity bias in schizotypy: A late positive potential study

Authors: A. WELBORN, D. BANUELOS, R. HURTADO, N. PAREDES, E. SANTANA, Y. ESCOBAR-VALENCIA, ***S. KANG**;
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Abstract: Negatively valenced information attracts more attention than positively or neutrally valenced information, and this greater sensitivity to negatively valenced information is referred to as the negativity bias. By measuring the event-related brain potentials, Ito and her colleagues (1998) and Hajcak and Olvet (2008) demonstrated that the negativity bias emerged during the early information processing. In these studies, participants were asked either to passively view or to respond to neutral, positive, and negative emotional pictures taken from International Affective Picture System (IAPS). Ito et al. and Hajcak and Olvet focused on the late positive potential (LPP) because it is a sensitive indicator for the emotional valence of stimuli. They reported that the LPP amplitude was much higher in the negative emotion condition compared to the neutral and positive conditions. The main purpose of the current study was to explore whether the same negativity bias would emerge among the individuals with a high degree of schizotypal traits. Given emotional dysfunction commonly observed among individuals with schizotypy and schizophrenia-spectrum disorders, we hypothesized that individuals with high schizotypal traits would not display the negativity bias. To test this hypothesis, a pool of 560 college students were screened with the Schizotypal Personality Questionnaire - Brief form. From that pool, the individuals whose scores comprised the top and bottom 10% were invited to participate. In an individual session, a 32-channel cap was applied on a participant's head following the 10/20 system. A total 120 pictures (40 positive, 40 negative, and 40 neutral photos) taken from the IAPS were presented for 2,000 ms with 1,500 ms intervals between images. Of the 22 participants who completed the study, usable data from 18 participants (9 high trait and 9 low trait) were included in the final analyses. The results of the mixed ANOVA analyses showed that there was a significant 3-way interaction effect of Group x Time x Emotion, $F(2, 32) = 4.284$, $p = .022$. This significant interaction effect implied that although no negativity bias emerged across the two groups in the early time window (400ms-2000ms), the negativity bias appeared in the low schizotypal trait group, but not in the high schizoptypal trait group, in the late window (2000ms-3000ms). The significance and implications of this lack of the negativity bias were discussed in terms of dysfunctional emotional behaviors in schizotypy.

Disclosures: A. Welborn: None. **D. Banuelos:** None. **R. Hurtado:** None. **N. Paredes:** None. **E. Santana:** None. **Y. Escobar-Valencia:** None. **S. Kang:** None.

Poster

235. Emotion: Positive and Negative Emotional States

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 235.05/S7

Topic: G.03. Emotion

Title: Psychological states and personality traits associated with laughter yoga — Evidence from a Japanese sample

Authors: *H. MIYATA, Y. SASE;
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Abstract: A good mount of empirical data suggest that laughter can cause desirable effects on the psychological, neurophysiological, and immune functions. Laughter yoga is a practice that systematically combines “unconditional laughter” (laughter elicited without any explicit reasons such as a comedy or a joke), breathing techniques of traditional yoga, and body movements. Originated in the 1990s, laughter yoga has gained increasing popularity in countries all over the world including Japan. Empirical data so far have shown that laughter yoga interventions can decrease state anxiety and cortisol levels and increase self-esteem, life satisfaction, natural killer (NK) cell activation, etc. The present study examined whether and how not only psychological states but also personality traits are associated with continued practice of laughter yoga among Japanese adults. Participants included 19 practitioners of laughter yoga (mean practice period: 3.1 years) and five non-practitioners as controls. Both prior to and immediately after a guided session of laughter yoga lasting for 75 minutes, these participants completed an identical battery of state questionnaires on positive/negative affect (PANAS) and anxiety (STAI-S). Participants also completed a questionnaire on personality traits (Big-Five Scale) after the practice session. For both groups of participants, positive affect significantly increased and negative affect and state anxiety significantly decreased after the session. Regarding negative affect, an interaction between group and time was significant, such that scores of negative affect were significantly higher in non-practitioners than in practitioners prior to the session but were statistically comparable between the groups after the session. In addition, the Agreeableness subscale scores from the Big-Five Scale were significantly higher in practitioners than in non-practitioners. These results support the notion that continued practice of laughter yoga can cause desirable effects not only on the psychological states when participating in the session but also on some dimensions of personality traits. Evidence from longitudinal studies as well as data at the neurophysiological and/or neuroendocrinological levels may further strengthen these views in future.

Disclosures: H. Miyata: None. **Y. Sase:** None.

Poster

235. Emotion: Positive and Negative Emotional States

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 235.06/S8

Topic: G.03. Emotion

Support: NIH R56 DK089100

Title: The relationship between stress and neural markers of food motivation among African American women: A pilot study

Authors: E. ONIANWA[1], *L. MARTIN[2], C. BEFORT[2], C. R. SAVAGE[3];
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Abstract: Obesity is associated with cardiovascular disease, diabetes, multiple types of cancer depression and anxiety. Currently 39.8% of adults in the United States are obese, however obesity rates differ by race and sex. Specifically, black women show the highest rates of obesity in the United States with 54.8% of black women compared to 38% of white women meeting clinical criteria for obesity. Genetics, environment and psychosocial factors such as stress have been proposed as contributors to this disparity. Stress has also been associated with differences in eating behaviors, such as emotional eating which can lead to obesity over time. This suggest that stress may increase the likelihood of an individual being obese by influencing food related decision-making and eating behaviors. The goal of this study is to examine the relationship between stress and the neural markers of food motivation. **Methods:** Participants included 19 African American females with a body mass index (BMI) between 18.5 and 40. Self-reported stress was measured using the Perceived Stress Scale and food motivation was measured by examining brain activation to visual food cues compared to non-food when participants had fasted for at least 4-hours. Whole-brain analysis was completed to identify regions that showed a correlation between self-reported stress and food motivation (i.e. Food - Nonfood). **Results:** The left middle frontal gyrus a region related to food motivation and self-regulation showed a positive correlation between food motivation and stress ($p<.001$ uncorrected). No significant differences were found in food motivation or stress between healthy weight and obese women. **Conclusion:** Overall, results suggest that increased levels of perceived stress may be related in increased activation in a brain region related to food motivation and self-regulation. This may indicate that higher levels of stress may be associated with greater engagement of self-regulation mechanisms in healthy weight and obese African American women.

Disclosures: E. Onianwa: None. **L. Martin:** None. **C.R. Savage:** None. **C. Befort:** None.

Poster

235. Emotion: Positive and Negative Emotional States

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 235.07/S9

Topic: G.03. Emotion

Support: University of Rhode Island Research and Innovation Student Research Grant

Title: The role of head trauma in emotion dysregulation among victims of physical intimate partner violence

Authors: *A. M. FRYC, R. NELSON, M. M. RISI, N. H. WEISS;
Univ. of Rhode Island, Kingston, RI

Abstract: Approximately 30% of all women will experience physical intimate partner violence (IPV) in their lifetime. IPV commonly results in physical injury to the head, which could cause significant impairment in psychological processes if a lesion (i.e. injury to an organ/tissue) is formed in the brain. For instance, patients with lateral frontal lesions in their brain show reduction in emotion, those with medial frontal injuries inhibit mood changes, and those with lateral prefrontal damage show irregular mood regulation (Paradiso et al., 1999). However, no investigations to date have examined whether head trauma stemming from physical IPV relates to the emotion dysregulation seen in this population. The aim of the current study is to examine whether there is an association between physical IPV and emotion dysregulation, and to assess whether head trauma resulting from IPV mediates the relationship between IPV and emotion dysregulation. 352 women (M_{age} = 36) were recruited from Amazon Mechanical Turk (MTurk), which is a reliable, online marketplace for work that requires human intelligence. Correlation results revealed significance between IPV and positive emotion dysregulation (r=.329, p &lt.001), negative emotion dysregulation (r=.274, p &lt.001), and head trauma (r=.114, p=.032). Mediation models provided evidence for the mediating role of head trauma to the relation between IPV and emotion dysregulation, with significant indirect effects for both positive (B=-.046, p &lt.001) and negative (B=-.054, p &lt.001) emotion dysregulation. In summary, these initial findings highlight the potential role of neurological precursors to emotion dysregulation. Results verified the significant relation between physical IPV and emotion dysregulation, as well as the mediating role of head trauma between physical IPV and emotion dysregulation. Additional research will be needed to further clarify the extent of the relation between IPV, head trauma, and emotion dysregulation.

Disclosures: **A.M. Fryc:** None. **R. Nelson:** None. **M.M. Risi:** None. **N.H. Weiss:** None.

Poster

235. Emotion: Positive and Negative Emotional States

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 235.08/S10

Topic: G.03. Emotion

Support: Center for Happiness Studies via the Center for Social Sciences at Seoul National University (No. 0404-20160001).

Title: Meaning vs. pleasure: Two types of happiness and the divergent evidence of molecular well-being

Authors: *S.-H. LEE[1], I. CHOI[1], S. W. COLE[2];
[1]Seoul Natl. Univ., Seoul, Korea, Republic of; [2]UCLA Sch. of Med., Los Angeles, CA

Abstract: Background Happiness has traditionally been thought to comprise two parts - pleasure (hedonia) and meaning (eudaimonia). Contemporary psychologists frame this idea in terms of subjective well-being (SWB) and psychological well-being (PWB). Even though SWB and PWB are highly correlated, recent studies suggest that they have distinct underlying neural correlates and genetic predispositions. Human social genomics has also identified divergent patterns of gene expression associated with SWB and PWB. In particular, sympathetic nervous system activity activates a Conserved Transcriptional Response to Adversity (CTRA) in immune cells - marked by up-regulation of proinflammatory genes and down-regulation of antibody-related genes. PWB (but not SWB) has been associated with reduced CTRA gene expression in several studies. However, most of these social genomics studies have been conducted in Western cultures, so it is unclear if these findings extended to non-Western cultures. Moreover, the moderation effect of age on the association between CTRA gene expression and well-being remains elusive. To this end, we examine CTRA profiles in association with PWB/SWB and the effect of age on the relation between them among Koreans. **Methods** Blood samples were collected from 152 healthy Korean adults (mean age=44.64). Ryff-psychological well-being scale, life satisfaction index and Positive and Negative Affect Schedule were measured. Whole RNA transcriptome profiles from peripheral mononuclear cells were obtained. Mixed effect linear model analyses examined the association between CTRA gene expression and measures of SWB, PWB (total scores and six domains) and the effect of age on them. **Results** CTRA gene expression was significantly downregulated in association with the PWB total score. Among the six domains of PWB, autonomy showed the strongest inverse correlation with CTRA profiles. This inverse association between CTRA and PWB strengthens with age. **Conclusion** The findings suggest that PWB, especially self-determination and independence (autonomy), can significantly impact immune cells gene expression among Korean population. Findings also suggest that ageing with autonomy may bring a health advantage in later life.

Disclosures: **S. Lee:** None. **I. Choi:** None. **S.W. Cole:** None.

Poster

235. Emotion: Positive and Negative Emotional States

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 235.09/S11

Topic: G.03. Emotion

Title: Electroencephalographic activity during breastfeeding in primiparous mothers

Authors: *J. P. GARCÍA-HERNÁNDEZ, M. PÉREZ-HERNÁNDEZ, R. M. HIDALGO-AGUIRE, C. GÓMEZ-NAVARRO, M. HERNÁNDEZ-GONZÁLEZ, M. A. GUEVARA; Univ. De Guadalajara, Guadalajara, Mexico

Abstract: The close bodily and affective interaction that occurs while mothers breastfeed their babies has beneficial effects on health, while also strengthening the maternal-infant bond. Mothers usually describe the experience of breastfeeding, in which the main source of sensory stimulation is the infant's sucking of the nipple, as pleasant, and there are reports that they show a relaxed electroencephalographic (EEG) pattern while nursing. Among the various brain areas that participate in modulating the breastfeeding are included the prefrontal and parietal cortices, which are activated during the detection and processing of sensory stimuli. Thus, the aim of the present study was to characterize the EEG activity of those cortical areas during breastfeeding and its relation to pleasant states in nursing mothers. A total of 18 healthy, right-handed primiparous mothers aged 20-35 participated after a maximum postpartum period of 9 months. EEG was recorded in the prefrontal and parietal cortices under two conditions: 1) while breastfeeding (3 minutes with each breast); and 2) while expressing milk mechanically using an electric extractor (also 3 minutes in each breast). During the breastfeeding condition, mothers reported a positive valence and presented higher absolute power (AP) in the delta and theta bands in the prefrontal areas, with lower AP in the alpha band in the parietal areas, compared to the mechanical extraction condition. These EEG results confirm the prevalence of slow frequencies during breastfeeding; a finding that could be associated with the greater pleasure and activation reported. These data suggest that the prefrontal cortex is involved in processing somatosensory stimulation and emotional responses during breastfeeding; while the parietal cortex participates in attention to stimuli.

Disclosures: **J.P. García-Hernández:** None. **M. Pérez-Hernández:** None. **R.M. Hidalgo-Aguire:** None. **C. Gómez-Navarro:** None. **M. Hernández-González:** None. **M.A. Guevara:** None.

Poster

235. Emotion: Positive and Negative Emotional States

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 235.10/S12

Topic: G.03. Emotion

Title: Incentive contrast in humans: Behavioral and electroencephalographic measures of reward relativity during a game-playing task

Authors: M. R. HARMON[1], E. L. STEWART[2], S. YUAN[3], M. SCHWARZMAN[3], ***H. C. CROMWELL**[4];
[1]Dept. of Psychology, [2]J.P. Scott Ctr. for Neuroscience, Mind and Behavior, [3]Psychology, [4]Psychology and J.P. Scott Ctr. for Neuroscience, Mind and Behavior, Bowling Green State Univ., Bowling Green, OH

Abstract: Incentive contrast is an effect of relativity with reward value changing from previous experience due to presence of available/comparable outcomes. Positive contrast is relative reward value increasing while negative contrast is relative reward value decreasing. These shifts can alter motivation and emotion and are compared to an experience with an outcome outside of a relative context. This study aims to extend the research on motivation and incentive contrasts by exploring the effects in humans while involved in a game-playing situation. We manipulated the sequence of experiences when playing a game in which the subjects have little to no experience. We predicted that the participants would be more motivated, feel more positive emotion and perform better when the easier version of the game follows the more difficult version. This is directly compared to another group experiencing the less difficult game repeatedly. In contrast, the participants will be discouraged in the difficult game following the easy game related to the frustration when encountering difficult task. Thus, we hypothesize that the performance will be worse in the hard game following the easy one than in the hard game repeated in series. Results support these predictions with poorer performance during the difficult game when it is preceded by the easier version and vice versa. Preliminary findings also suggest a gender effect with females showing stronger positive and negative contrast effects. The data obtained for self-report of motivation or emotion were not as robust showing less intense shifts in these dependent variables. The project goals include examining measures of electroencephalography during the game-playing experiences to decipher neural oscillatory activity related to alterations in behavior, motivation or emotion related to exposure to various sequences of game difficulty level.

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Poster

235. Emotion: Positive and Negative Emotional States

Location: Hall A

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Program #/Poster #: 235.11/S13

Topic: G.03. Emotion

Support: NRF KOREA Grant 2017R1D1A1A09000664
NRF KOREA Grant 2017M3C7A1041823

Title: An implicit association learning of smart-device pictures and emotion words influences EEG oscillations and evaluations of real-life smart technology news reports

Authors: *H.-J. KIM[1], T. LEE[2], Y. JEONG[1], S.-P. KIM[2], S. KIM[1];
[1]Korea Univ., Seoul, Korea, Republic of; [2]Ulsan Natl. Inst. of Sci. and Technol., Ulsan, Korea, Republic of

Abstract: Smart devices are becoming more popular worldwide. As dependency on smart devices such as smartphones and tablets increases, there has been increased recognition of problems in association with overuse of such devices. Various attempts have been made to develop effective treatment strategies to alleviate overuse. This study developed an implicit association learning task in which pictures of smart devices were paired with either positive or negative emotion words. We tested whether individuals showed different subjective and EEG responses to real-life news reports featuring smart technologies, depending on the emotion words associated with smart devices during the learning task. Fifty-two healthy young adults participated in the current study. They were randomly assigned to either the benign and malignant group and performed an implicit learning task of picture-word association. In the association learning task, a picture appeared in the computer screen and participants clicked the picture to find a word. Participants were asked to memorize the word. For the benign group, the emotion words were typically positive emotion words; for the malignant group the words were negative words. After the association task, participants watched a total of 6 positive and negative news reports including those featured beneficial and harmful uses of smart technologies. Behavioral measures of emotional valence, arousal, societal impact, and personal impact of the news were obtained on a 7-point scale after each news report. Sixty-four channels EEG responses were also obtained from all participants. Spectral powers of EEG oscillations within the delta, theta, alpha and beta ranges were analyzed and compared between groups. Behavioral results revealed that the malignant group relative to the benign group tended to evaluate the news report featuring the harmful use of smart device with less negativity, reduced societal, and reduced personal impact. EEG oscillations in alpha frequency band (8-13Hz) also differed between the groups. This study presents evidence to suggests that implicit association of smart-

device and emotion word may influence electrophysiological responses and subjective evaluation of subsequently presented news reports regarding the use of smart technologies.

Disclosures: H. Kim: None. **T. Lee:** None. **Y. Jeong:** None. **S. Kim:** None. **S. Kim:** None.

Poster

235. Emotion: Positive and Negative Emotional States

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 235.12/S14

Topic: G.03. Emotion

Support: NSERC

Title: Effects of affective valence and attentional relevance on visually evoked event-related potentials and event-related spectral perturbations

Authors: *R. A. HICKS, W. R. STAINES;
Kinesiology, Univ. of Waterloo, Waterloo, ON, Canada

Abstract: Understanding affective valence processing both between and within individuals has become more important with increasing prevalence in mood disorders such as anxiety and depression. Although establishing differences between clinical and non-clinical populations is apt, there remain questions of how valence processing is influenced by attention in a healthy population. By understanding these relationships, future research can discern potential differences in these processes between clinical and non-clinical populations. Previous literature has shown mixed results for N2 and P3 effects of valence, which may be due to interaction of task demands and attention. Therefore, this study aimed to test the effects of valence relevance in responding during an affective oddball task on ERP and ERSP responses. Participants viewed a visual oddball task using positive, negative and neutral valence images with 30% of images being non-neutral. Participants were asked to respond by pressing a button when they saw either a positive or negative stimulus. They also viewed the images in a passive condition in which they did not respond. Results showed that frontal N2 and parietal P3 responses had a significant main effect of valence on ERP amplitude such that negative was larger than positive. There was a significant main effect of relevance such that passive stimuli were significantly decreased P3 amplitude compared to ignored and target valence stimuli. ERSP results showed increased theta in valence stimuli during target conditions in the 200-400ms time window compared to passive valence stimuli. This research suggests that there is a negativity bias in valence processing that is not influenced by the attentional relevance of the stimulus.

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Poster

235. Emotion: Positive and Negative Emotional States

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Program #/Poster #: 235.13/S15

Topic: G.03. Emotion

Support: NIH R01MH11255801

Title: Distracted by affective pictures: Neural mechanism revealed by multivariate analysis

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Abstract: Affective pictures are highly potent distractors. In this study we examined the impact of picture valence on task-relevant visual processing and the underlying neural mechanisms. Simultaneous EEG-fMRI were recorded while participants detected instances of coherent motion in a random dot kinematogram (RDK) overlayed on IAPS pictures (pleasant=erotic couples, neutral=workplace people, and unpleasant=bodily mutilations). RDK and IAPS pictures flickered on and off at different frequencies, evoking two independent steady-state visual evoked potentials (ssVEP). Applying support vector machines to BOLD responses in ventral visual cortex and MT cortex we found the following results. First, decoding accuracy of both pleasant-vs-neutral and unpleasant-vs-neutral distractors was above chance level in ventral visual cortex, at 62.6% and 59.4% respectively; pleasant-vs-neutral decoding accuracy was marginally higher than unpleasant-vs-neutral decoding accuracy ($p=0.08$). Second, across subjects, decoding accuracy of unpleasant-vs-neutral distractors was negatively correlated with the correctly identified instances of coherent motion ($p=0.01$), namely, the higher the decoding accuracy the lower the correctly identified instances of coherence motion; decoding accuracy of pleasant-vs-neutral distractors, however, was not associated with behavioral performance ($p=0.9$). Third, in MT cortex, decoding accuracy of pleasant-vs-neutral and unpleasant-vs-neutral distractors was also above chance level, at 71.2% and 64.5% respectively, with pleasant-vs-neutral decoding accuracy significantly higher than unpleasant-vs-neutral decoding accuracy ($p=0.0004$). Fourth, neither the pleasant-vs-neutral decoding accuracy nor the unpleasant-vs-neutral decoding accuracy in MT cortex was found to be predicting behavioral performance ($p>0.05$). In summary, these results demonstrated that (1) although pleasant distractors were better represented in both ventral visual cortex and MT cortex than unpleasant distractors, it was the unpleasant distractors that had a stronger adverse influence on behavior and (2) although MT cortex was the neural substrate underlying the task-relevant visual processing, it was the ventral visual cortex where the processing of unpleasant distractors adversely impacted behavior.

Disclosures: K. Bo: None. **N.M. Petro:** None. **A. Keil:** None. **M. Ding:** None.

Poster

235. Emotion: Positive and Negative Emotional States

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 235.14/S16

Topic: G.03. Emotion

Support: Grant-in-Aid for Young Scientists (B) (KAKENHI 23700306)
Grant-in-Aid for Young Scientists (A) (KAKENHI 25700012)

Title: Mean diffusivity of the dopaminergic system associated with subjective well-being in young healthy adults

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Abstract: Although health is not merely the absence of disease, the positive aspects of mental health have been much less investigated than negative aspects. Subjective well-being (SWB) is one of the indicators of positive psychology such as happiness, life satisfaction, and positive affect. Some previous studies which investigated the neural correlates of SWB indicated that the dopaminergic system, including the striatal areas contributes to SWB. However, to our knowledge, very few studies have examined the association between brain microstructure properties detectable by diffusion tensor imaging (DTI) and SWB. The aim of this study is to investigate the relationship between mean diffusivity (MD), a measure of DTI, and degrees of SWB measured using a questionnaire. The World Health Organization subjective well-being inventory (SUBI), which is a self-report questionnaire measuring an individual's mental healthfulness from positive (PA) and negative (NA) affects respectively was used in this study. The voxel-based analyses were performed to investigate the association between MD and the PA

or NA scores of SUBI, across the brain in young healthy right-handed adults (695 males and 514 females; age: 20.7±1.8 years). These analyses were performed using multiple regression models with sex, age, the Raven's advanced Progressive Matrix score, total intracranial volume, and PA or NA score as covariates. Correction for multiple comparisons was performed using threshold-free cluster enhancement (TFCE), with randomized (5000 permutations) nonparametric permutation testing. We applied a family-wise error (FWE)-corrected threshold of $P<0.05$. We found that better SWB states (in both positive and negative aspects) were associated with lower MD in areas related to the dopaminergic system including the right putamen, pallidum, thalamus, caudate, and insula. The results showed that individual SWB is reflected in the variability in the brain microstructure properties of areas in the dopaminergic system.

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Poster

235. Emotion: Positive and Negative Emotional States

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Topic: G.03. Emotion

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Shimadzu science Foundation
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Title: Combined representation of emotional salience and decision signals in primate dorsal raphe nucleus

Authors: *M. YASUDA[1], Y. UEDA[1], K. NAKAMURA[2];
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Abstract: While our decision making is often influenced by emotional context, the degree and types of influence may be controlled; one may or may not perform a task correctly even under stress. However, the neuronal mechanisms underlying this process are largely unknown. Neuronal activity in the dorsal raphe nucleus (DRN), the center of serotonergic system, is modulated by emotional context created by Pavlovian conditioning. Manipulation of serotonin induces distinct behavioral effects under distinct emotional state. We therefore hypothesized that single DRN neurons would be involved in both emotional coding and decision making. To this end, we recorded single DRN neurons' activity while monkeys performed a reversal choice task under different emotional contexts.

In the task, after fixation on the central fixation point (FP, 1-1.5s), two different visual cues were presented simultaneously in the left and right of the FP. After further fixation (0.5s), monkeys (n=2) chose one of them by making a saccade, followed by a reward for one (correct), but not for the other (wrong). During a block of trials, the reward-associated cue was identical, but it switched to the other one when the proportion of the correct choice reached the criterion. During the block, one of three pre-learned 'emotional CS', associated with reward (appetitive), tone (neutral), or air puff (aversive), was chosen and was repeatedly presented during the inter-trial-interval. This manipulation successfully created the situation where the task was performed under distinct emotional context, confirmed by significant modulation in autonomic responses: larger pupil diameter and heart rate, and in behavior: more frequent fixation breaks in the presence of an aversive CS.
Among 176 task-related neurons, 82 showed significant modulation in the pre-cue period depending on the emotional context. Majority (51/82) were more excited during the appetitive and/or aversive context. The tendency of increased or decreased firing during the pre-cue fixation period for appetitive or aversive-preferred neurons, respectively, indicates the consistent emotional coding across decision-making tasks and conditioning (Hayashi et al. 2015). More than half of DRN neurons (99/176) represented monkeys' choice (correct or wrong) before target onset. They tended to be more excited in correct than wrong choice trials (74/99). They were also modulated by CS (60/99), and majority of them showed stronger activity for appetitive and/or aversive than other CS conditions (42/60). Altogether, our data showed that sensitivity of the DRN neurons to the emotional context also influences the ongoing behavior.

Disclosures: M. Yasuda: None. **Y. Ueda:** None. **K. Nakamura:** None.

Poster

235. Emotion: Positive and Negative Emotional States

Location: Hall A

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Program #/Poster #: 235.16/S18

Topic: G.03. Emotion

Title: Interaction between the endocannabinoid and oxytocin system within the PFC: Implication for social behavior

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Abstract: Cannabis is the most used psychoactive drugs with an estimates 125-227 million consumers all over the world. The most powerful component of cannabis is the Δ^9-Tetrahydrocannabinol (Δ^9-THC), which acts as agonist on cannabinoid receptor 1 (CB1R) modulating several functions especially social and emotional memories. In fact, CB1R is mainly expressed in the Prefrontal Cortex (PFC), which plays a pivotal role in modulating social

abilities. In particular, recent studies have been demonstrated the presence of CB1R in astrocytes, but its role in social interaction and emotion discrimination remains largely unknown. Indeed, social cognition is a fundamental ability that influences development, survival and evolution of animals. Human social cognition is assessed measuring the ability to recognize others' emotions, a function that remains elusive for laboratory animals. In our lab, we revealed that mice are able to discriminate unfamiliar conspecifics based on emotional states. In order to investigate the role of astrocytic CB1R in modulating social interaction, we selectively removed CB1R in prefrontal astrocytes in CB1 floxed mice and tested them in our new social paradigms "emotion discrimination tasks". Preliminary data demonstrated that removal of CB1R in prefrontal astrocytes produces deficit in social abilities, decreasing time spent to interact in both social interaction and emotion discrimination tasks. Nevertheless, the exact mechanisms involved in emotion discrimination in mice are still poorly investigated. In particular, oxytocin has been proposed as a candidate in modulating social interaction in both rodents and mice. In order to understand if the endocannabinoid system and the oxytoninergic system might interact in emotion discrimination, we treated CB1 floxed mice with acute and chronic intranasal oxytocin. Our findings highlight how endocannabinoid system and oxytoninergic system may contribute to develop social impairments and establish our model as a valid experimental tool to investigate the role of astrocytes in sociability.

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Poster

235. Emotion: Positive and Negative Emotional States

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Program #/Poster #: 235.17/T1

Topic: G.03. Emotion

Support: NIMH-R21MH112539

Title: Neural mechanisms of affective states in non-human primates

Authors: *J. A. MUNOZ[1], M. YOUNG[1], E. BLISS-MOREAU[2], P. RUDEBECK[1];
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Abstract: Mood disorders are highly prevalent and impact the lives of millions worldwide. Despite this, little is known about the dynamic functions of the neural networks that contribute to these disorders. Studies of healthy individuals and those with depressed mood implicate a network of areas centering on ventral anterior cingulate cortex (ACC) and amygdala in the control of long-term changes in affect. Additionally, studies have found aberrant activity between the two areas in cases of severe depression in humans. In order to gain a greater understanding of these networks and neural mechanisms engaged, we set out to determine how

single neurons and local field potentials in macaque subcallosal ACC and amygdala are affected when positive and negative affective states are induced. Affective states were induced in two adult rhesus macaques (one male, one female) by showing then six hundred, 30-second video clips over the course of 12 sessions (50 per session). Video stimuli ranged in affective valence from extremely negative to extremely positive and had previously been validated by Bliss-Moreau et al., 2013. For example, videos characterized as positive may include monkeys engaged in grooming while those characterized as negative may include displays of aggressive behavior such as fighting. Furthermore, each video was ranked on level of arousal, dominance, submission, closeness, and novelty as well as number of interactions between monkeys in the video clips. Induced affective states were characterized by indexing cardiac sympathetic and parasympathetic activity. Taking this approach, we successfully replicated the results of Bliss-Moreau, et al., (2013). Specifically, we found that there was an increase in sympathetic activity as the valence of the video went from positive to negative, while parasympathetic activity decreased. We interpret this as showing the induction of affective states as subjects viewed the different 30-second video clips. While monkeys viewed the video clips, 16-channel linear arrays were placed in subcallosal ACC and amygdala and neural activity was recorded simultaneously with ECG and ICG. Our study has the potential to reveal the neural mechanisms engaged in ventral ACC and amygdala during temporally extended affective states. More generally, they provide a translation model in which the underlying mechanisms that control mood-like affective states in non-human primates can be elaborated to gain insights into human mood disorders.

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Poster

235. Emotion: Positive and Negative Emotional States

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Program #/Poster #: 235.18/T2

Topic: G.03. Emotion

Support: DA014339
F32 MH115653

Title: Infralimbic cortex ensembles encode hedonic valence

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Abstract: The hedonic impact of stimuli powerfully impacts behavior. Here, we examined how infralimbic cortex (IL) neural ensembles encode learned and innate hedonic value. In Exp. 1, we tested if IL neuron encoding of hedonic value shifts over conditioned taste aversion (CTA)

learning and extinction. Microelectrode arrays were placed in the IL and taste reactivity (TR) and neuronal activity (55 cells/5 rats) was recorded during intraoral (IO) infusions of 0.5M sucrose across testing. Sucrose elicited appetitive TR in naïve rats but evoked aversive TR after CTA was established ($p<0.01$), and extinction restored the appetitive value of sucrose ($p<0.001$). Further, IL signaling tracked learned hedonic value. In naïve rats, 20 neurons inhibited to sucrose infusion, 5 cells were excited, and 30 neurons were nonphasic (no change in cell firing). During CTA, more nonphasic activity emerged (38 cells); the remaining phasic neurons showed a mixed excitatory-inhibitory profile (6 excitatory, 7 inhibitory). During extinction, a small increase in excitatory tone was observed (10 cells excited; 4 inhibited) while 32 neurons were nonphasic. In Exp. 2, we extended that work and employed deep brain *in vivo* calcium imaging to measure large population neuronal ensemble activity to identify IL ensembles that uniquely process either innately rewarding sucrose or aversive quinine (unconditioned) or encoded both types of hedonic stimuli. Rats received IO infusions of 0.5M sucrose followed by 0.05mM quinine and calcium signal (197 cells/4 rats) and TR was recorded. Sucrose elicited appetitive TR, while quinine evoked aversive TR ($p<0.001$). Sucrose mostly inhibited calcium activity in IL cells (83 inhibited; 37 excited), while quinine tended to excite IL neurons (45 excited; 28 inhibited). We also examined neuronal ensembles that were phasic to *both* sucrose and quinine. Here, a distinct IL ensemble appears to track hedonic value as these cells are inhibited to sucrose, but excited to quinine (24 neurons) while another ensemble may encode salience (18 neurons excited to both sucrose and quinine; 12 inhibited to both). Together, these data indicate that the IL uniquely processes innate and learned hedonic value. An innately rewarding or aversive stimulus tended to inhibit or excite IL neurons, respectively (as measured using electrophysiology or calcium imaging methods). In contrast, learned aversion during CTA resulted in a loss of phasic activity, indicating that the IL no longer processes the hedonic value of sucrose during CTA. However, elevated excitatory tone is present during extinction, indicating that the IL may activate to suppress learned aversive responses.

Disclosures: **S.W. Hurley:** None. **R.M. Carelli:** None.

Poster

235. Emotion: Positive and Negative Emotional States

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Topic: G.03. Emotion

Support: JSPS KAKENHI Grant Numbers JP16K21509,JP19K03388

Title: Segregated and overlapped processing of social and reward information in primate amygdala

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Abstract: Social and reward information are independent in nature while these two are closely related. However, it is not clear whether they are computed separately or conjointly in the brain. To this end, we developed a saccade task in which the monkey made saccades under different social and reward context. After fixation on a central fixation point (FP), a target dot was presented on left or right of the FP to which it made a saccade to obtain a liquid reward. During a trial, one of 8 images with 2 attributes: social reality; a monkey or cartoon face, and reward; large or small, was presented twice: after fixation onset (predictor) and just before target presentation (direct cue). The monkey showed larger pupils (a reflection of sympathetic arousal) in response to monkey faces than to cartoon faces, and preferred large rewards to small rewards, confirmed in a separate choice task, indicating that the monkey recognized these 2 attributes. It has been reported that the amygdala neurons signal social (in the basolateral nuclei) as well as reward (in the basolateral and centromedial nuclei) information. Thus we analyzed activity of single neurons in the lateral (LA, n=31), basal (BA, n=38), and central (CE, n=39) nuclei of amygdala. In LA, population response of 11/31 neurons (35%) showing excitatory response to the predictors of social reality is stronger to monkey faces than to cartoon faces. Population response of 14/31 neurons (45%) showing inhibition to reward is stronger to a large-reward than to a small-reward. Eight of 31 (26%) showed discriminative response to both face types and amount of rewards, indicating that some LA neurons convey both social reality and reward information. In BA, population response of 11/38 neurons (29%) showing excitatory response to the predictors of reward is stronger to the predictor of a large-reward than a small-reward. Population response of 6/38 neurons (16%) showing inhibition after the predictors of reward is stronger to a large-reward than a small-reward, indicating reward information processing. In CE, population response of 8/39 neurons (21%) showing inhibition after the predictors of reward is stronger to a large-reward than a small-reward, indicating reward information processing. These results indicate that, in the primate amygdala, reward information is processed across different subnuclei while social information is processed in a restricted part of the subnuclei.

Disclosures: K. Kuraoka: None. **K. Nakamura:** None.

Poster

235. Emotion: Positive and Negative Emotional States

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Topic: G.03. Emotion

Support: R01MH101214 (NIH)
RGP0015/2016 (Human Frontier Science Program

Title: Central amygdala somatostatin neurons modulate both reward and aversive learning

Authors: *T. YANG[1], K. YU[3], X. ZHANG[3], B. LI[2];
[1]Cold Spring Harbor Lab., New York, NY; [2]Cold Spring Harbor Lab., Cold Spg Hbr, NY; [3]Cold Spring Harbor, New York, NY

Abstract: The central amygdala (CeA) has been implicated in fear learning as well as reward learning. However, how CeA participates in these different functions remains largely unknown. Here, we used *in vivo* imaging combined with optogenetics to study the functions of somatostatin expressing (SOM+) CeA neurons during both reward and aversive learning in behaving mice. We find that there are two functionally distinct SOM+ subpopulations, which process appetitive and aversive stimuli separately, and that inactivation of SOM+ CeA neurons impairs both aversive and reward learnings. Further anatomical and functional studies suggest that these functions of SOM+ CeA neurons are mediated by their specific downstream targets. Overall, our results reveal novel circuit mechanisms of CeA modulation of both aversive and reward learning.
*These authors contributed equally to this work

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Poster

235. Emotion: Positive and Negative Emotional States

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Program #/Poster #: 235.21/T5

Topic: G.03. Emotion

Support: 1R01MH114882-01
5R01MH104559-04
5R01MH090264-07

Title: The posterior cortical amygdala is required for the full expression of aggressive behavior

Authors: *A. V. AUBRY, M. FLANIGAN, H. ALEYASSIN, L. LI, K. LECLAIR, L. PARISE, S. RUSSO;
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Abstract: Aggression is a ubiquitous behavior amongst all mammals which is necessary for the acquisition of mates, territory and food. Numerous studies have demonstrated that activation of the VMHvl is required for the initiation of aggressive behavior. More recent studies have identified nuclei within the ventral midbrain and basal forebrain that control the valence of male-typical aggressive interactions. However, it remains relatively unknown how odor cues interact with the internal state of the animal to elicit aggression. We thus performed the resident intruder test (RI) and categorized mice as being in an aggressive state (AGG) or a non-aggressive state

(NON) based on their behavior in the RI test. To determine which regions of the olfactory cortex are active during aggressive behavior, we conducted a c-fos mapping study using the iDISCO brain clearing method. We found that numerous brain regions in the olfactory cortex demonstrated higher levels of c-fos in AGGs compared to NONs. A subsequent network analysis on these brain regions demonstrated that the posterior cortical amygdala (COAp) was a key node in the olfactory network. To follow up on this observation, we injected a CAMKIIα-DREADDi into the COAp of AGGs to inhibit glutamatergic cell activity during the RI test. Following injection of CNO, we observed a significant increase in attack latency with a concomitant decrease in attack duration. These finding suggest that glutamatergic activity in the COAp is required for the full expression of an aggressive behavioral state. Current studies are underway to determine the afferent and efferent projections of the COAp which are involved in promoting aggressive behavior.

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Poster

235. Emotion: Positive and Negative Emotional States

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Topic: G.03. Emotion

Support: NIH D43

Title: The establishment sexual defeat rat model

Authors: *L. L. QULU[1], A. WILKINS[2];
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Abstract: South Africa has been named the “rape capital of the world” with 110 rape cases reported daily. Sexual violence against women is a major international public health problem and a violation of women's human rights. However, there is a dearth of viable data on factors perpetuating sexual violence and whether aggressive behaviour or factors leading to aggressive behaviour such as social isolation play a role in perpetuating sexual violence. Social isolation has been shown to dysregulate the HPA axis which plays a pivotal role in the regulation of both corticosterone and oxytocin. Furthermore, there is a dearth of animal models designed to mimic sexual violence in a controlled laboratory environment. Therefore, the aim of this study was to create a model of sexual defeat using juvenile Sprague-Dawley rats (SD). Virgin male SD rats were exposed to either group housing or social isolation concurrently for seven days. After this the resident-intruder test was used to assess aggressive behaviour. These males were then

exposed to females in oestrus, and upon the second intromission the female in oestrus was removed and replaced with a female not in oestrus. This model was repeated for a total of four days after which the resident-intruder paradigm was repeated. After the resident-intruder paradigm, the social dominance tube test was used to assess social deficits in the male rats. Our findings show that social isolation resulted in increased sexual aggression towards females which also culminated increased aggression towards male intruders and social deficits. Interestingly, group housed males exhibited less aggression, and tended to use more coercive actions towards females. These findings were confirmed by high oxytocin levels observed in the group housed males in comparison to the isolated sexual defeating males. In conclusion, this model will allow for the study of sexual aggression in both man and woman in a controlled environment, with a focus on physiological changes which may occur in perpetrators.

Disclosures: L.L. Qulu: None. **A. Wilkins:** None.

Poster

235. Emotion: Positive and Negative Emotional States

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 235.23/T7

Topic: G.03. Emotion

Title: Hangry - An analysis of its behavioural pharmacology

Authors: *T. A. HORMAN[1], B. MELANSON[1], M. SCOTT[1], F. LERI[2];
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Abstract: Background: disorders characterized by dysfunctions in glucose metabolism are often comorbid with negative mood. The glucose antimetabolite 2-deoxy-D-glucose (2-DG) causes a stress response characterized by hypoglycemia and negative affect. The current project in laboratory animals is designed to investigate the impact of acute and repeated hypoglycemic stress on physiological and hedonic responses.
Methods: in Experiment 1, male Sprague-Dawley rats were trained to self-administer the nutritional reinforcer high-fructose corn syrup (HFCS) via lever-pressing for an oral infusion for 14 days and then the effects of 2-DG (200 and 300 mg/kg; SC) were tested. In Experiment 2, 2-DG (200 and 300 mg/kg; SC) was injected immediately prior to acquisition of self-administration using progressive-ratio and fixed ratio schedules. Experiment 3 assessed whether 2-DG (200 and 300 mg/kg; SC) could alter appetitive oral-facial responses associated with HFCS using taste-reactivity. Finally, Experiment 4 measured the effects of repeated 2-DG administration (200 and 300 mg/kg; SC; one injection per day for 10 days) on blood glucose and corticosterone (CORT).
Results: Experiment 1 demonstrated that 2-DG suppressed lever-pressing for HFCS for up to 6 days. Experiments 2 and 3 ruled out the possibility that this suppression was due to a context-

induced malaise or to the development of taste aversion. In Experiment 4, repeated administration of 2-DG blunted the normal blood glucose response.
Conclusions: These results suggest that hypoglycemia can induce a lasting anhedonic state characterized not only by negative affect, but also by physiological dysregulation and impaired consummatory responses to incentive stimuli.

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Poster

235. Emotion: Positive and Negative Emotional States

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Title: Boredom-like behavior in an unadorned mouse room

Authors: *Y. YAWATA, Y. IKEGAYA;
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Abstract: We often get bored when we have nothing to do or when we continue to do a simple task. Boredom is defined psychologically as “the aversive state of wanting, but being unable, to engage in satisfying activity” (Eastwood et al., 2012). To our knowledge, the neural circuit mechanism by which we get bored is yet to be determined. Recently, the neural correlates of boredom have been investigated using functional magnetic resonance imaging (fMRI). In human studies, however, the invasive method can hardly be used, including single-neuron recordings and manipulation of neuronal activity. To elucidate the neural mechanism of boredom in the single-cell level and in the neural circuit level, we sought to establish a behavioral paradigm to quantify the extent of boredom of mice. In a human study, people do not enjoy spending several minutes in an unadorned room by themselves with nothing to do but think. Moreover, many of them preferred to give electric shocks to themselves in place of being left alone with their thoughts (Wilson et al., 2014). We hypothesized that mice, like human, choose giving themselves aversive stimuli if they get bored. To test this hypothesis, we put a male mouse for 15 min in an empty chamber that contained nothing but a nose-poke hole. When the mouse poked its nose into the hole, an air-puff stimulus, which was naturally aversive for mice, was given to it. We defined this nose-poke behavior as a “boredom-like” behavior. And its repeat number was used as an index of boredom of mice. For control conditions, we used an enriched chamber that contained multiple toys, such as a ladder and a seesaw. We found that mice exhibited more nose-

poke behaviors in the empty chamber than in the enriched chamber. In addition, mice in the empty chamber exhibited more jumping behaviors onto the chamber walls, which are considered behavioral trials to escape out of the chamber. These results suggest that mice get bored in an impoverished environment and try to avoid the aversive situation that has nothing to do.

Disclosures: Y. Yawata: None. **Y. Ikegaya:** None.

Poster

235. Emotion: Positive and Negative Emotional States

Location: Hall A

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Program #/Poster #: 235.25/T9

Topic: G.03. Emotion

Title: Back to nature: Icariin increases emotional resilience in male Long-Evans rats

Authors: *M. BARDI, K. JOHNSON, K. NWACHUKWU, E. RHOADS, S. MEEK; Randolph-Macon Col., Ashland, VA

Abstract: Icariin, a major constituent of flavonoids isolated from *Epimedium brevicornum*, has been used for centuries in traditional Chinese medicine to treat stress and anxiety. Central mechanisms of activations for icariin have been recently identified, including a significant increase in brain-derived neurotrophic factor (BDNF) and an altered expression of hippocampal glucocorticoids receptors (GC) after stress (Wei et al., 2016). In this study 29 male Long-Evans rats were randomly assigned to three treatment groups. Nine rats were used as control, 10 rats were administered low doses of icariin (40mg/kg) via dietary supplement, and 10 rats were given double that dose (80mg/kg). All animals were exposed to chronic unpredictable stress for four weeks. Stressors included predator calls, tail pinching, and predator odors. Stressors were randomized for the different days. At week four, animals were tested in an open field (OF) with a novel object (NO) placed in the center. Hormonal levels were monitored throughout the study, and before and after the NO test, by collecting fecal samples and assaying for metabolized corticosterone and dehydroepiandrosterone (DHEA) peripheral levels. BDNF immunoreactivity in the hippocampus were assessed by immunohistochemistry. Results showed that animals treated with high dose of icariin had higher levels of DHEA and lower levels of CORT during the NO test than both control and low-dose groups. They also showed higher frequencies of interactions with the NO, thus indicating that these animals were able to cope effectively with the test in the OF. Finally, icariin significantly increased BDNF immunoreactivity in the hippocampus in both low-dose and high-dose groups. Throughout the study, CORT increased more rapidly in the control animals than in the other two groups. Overall, our study revealed a significant improvement in the neuroendocrine and behavioral coping skills of chronically stressed animals when treated with icariin.

Disclosures: **M. Bardi:** None. **K. Johnson:** None. **K. Nwachukwu:** None. **E. Rhoads:** None. **S. Meek:** None.

Poster

236. Depression: Pathology

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 236.01/T10

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: NCCIHATR010091692
VA merit BX001149
VA research career scientists BX 004475

Title: N-3 polyunsaturated fatty acids promote neurotrophic factor production in human nsc-derived glial cells from subject with major depressive depression

Authors: *J. YU[1], J. WANG[2], R. PERLIS[2], M. M. RASENICK[3];
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Abstract: Major depression disorder is a leading cause of disability worldwide. Unfortunately, about one third of patients treated with conventional antidepressants do not experience a response. The evidence from epidemiological, laboratory, and randomized placebo-controlled trials suggests supplementation with n-3 PUFAs may provide a treatment option for depression. The mechanism underlying n-3 PUFAs alleviating depression is remain unknown. Studies have suggested that hypofunction of glial cell were associated with depression progression. Some antidepressants may recover glia function, like BDNF production. In the study, iPSCs were generated from fibroblasts of two depression patients; one was responded to SSRI treatment, other one was resistant to SSRIs treatment. Differentiated glial cells from induced neuron stem cells were verified by GFAP. The induced glial cells treated with eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and stearic acid (SA). The increase of BDNF and GDNF production was seen in the induced glial cell. SA did not alter the BDNF and GDNF. The activity of CREB (phosphorylated CREB) was also increased by DHA and EPA treatment in these cells, but not by SA. These effects of n-3 PUFAs on neurotrophic factors and CREB activity did not show different between the induced glial cells from patient who was sensitive and resistant to SSRIs. Furthermore, when glial cells were treated with n-3 PUFAs, the cAMP antagonist, RP-cAMP, did not diminish the activity of CREB induced by DHA and EPA. RP-cAMP did not alter the BDNF and GDNF production promoted by DHA and EPA. These suggested that n-3 PUFAs could stimulate the glial cell function from either SSRIs sensitive or

resistant patient, increasing production of neurotrophic factors, which may contribute to n-3 PUFAs benefit effect for depression patients. DHA and EPA elevated the activity of CREB with a manner of independent of cAMP signaling.

Disclosures: **J. Yu:** None. **J. Wang:** None. **R. Perlis:** None. **M.M. Rasenick:** None.

Poster

236. Depression: Pathology

Location: Hall A

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Program #/Poster #: 236.02/T11

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: R41 MH113398
NIH R01AT009169
VA Merit BX001149
VA Research Career Scientist BX004475

Title: Polyunsaturated fatty acids affect a depression biosignature in lymphoblast cell lines from depressed human subjects

Authors: *P. CHUKAEW[1,2], M. M. RASENICK[2,3,4];
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Abstract: Omega-3 polyunsaturated fatty acids (n-3 PUFAs) are well-known inflammatory regulators and also modulate brain function and development. Peripheral n-3 PUFA levels in depressed patients negatively correlate with symptom severity, suggesting a relationship between n-3 PUFAs and depression. Furthermore, n-3 PUFA consumption has been reported to have antidepressant effects. Hypotheses underlying its mechanisms include neuroendocrine and pro-inflammatory modulation. However, the cellular and molecular mechanisms of n-3 PUFAs are little known. In this study we hypothesize that n-3 PUFAs directly affect the membrane micro-domains known as lipid rafts and G protein ($G\alpha_s$) translocation from those rafts alters the downstream signaling cascades related to neuroendocrine and pro-inflammatory functions. Human lymphoblast cell lines were obtained from NIMH from healthy controls, depressed subjects responding to initial citalopram (SSRI) therapy and subjects unresponsive to citalopram (pulled from the STAR*D study). Basal gene expression was measured in each cell line using RT-PCR. Then, cells were treated with n-3 PUFAs ± escitalopram for 3 days. Effects of n-3 PUFA treatment on $G\alpha_s$ translocation from lipid rafts, $G\alpha_s$ activation of adenylyl cyclase and the attendant increase in cAMP and expression of proteins related to lipid rafts and G protein signaling were determined. Alpha screen was used to measure cAMP accumulation and

biochemical fractionation was employed to measure $G\alpha_s$ translocation. Initial gene expression profiles for signaling proteins and raft proteins are different among the three groups, and n-3 PUFAs change the membrane micro-domains and related downstream signaling cascades in lymphoblasts from both healthy control and depressed subjects, including those resistant to SSRIs. Taken together these data suggest that lymphoblasts might be a good model for human depression and n-3 PUFAs could be a good supplement or adjuvant for the treatment for depression.

Disclosures: P. Chukaew: None. **M.M. Rasenick:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pax Neuroscience.

Poster

236. Depression: Pathology

Location: Hall A

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Program #/Poster #: 236.03/T12

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: NIH R01 AT009169
VA Merit BX001149
VA Research Career Scientist BX004475

Title: Impairment of lipid raft Gs signaling persists following antidepressant withdrawal

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Abstract: Termination of antidepressant therapy has negative consequences for many patients. While the symptoms of antidepressant withdrawal are widely recognized, the molecular processes that underlie them are not well characterized. We show that certain aspects of Gs signaling remain suppressed following antidepressant withdrawal, even after others have reverted to baseline.
Lipid rafts are membrane microdomains characterized by high cholesterol content, detergent insolubility, and unique signaling characteristics. Antidepressant treatment causes translocation of Gs protein from lipid rafts, to non-raft membrane regions. This results in augmented Gs signaling, including facilitated activation of adenylyl cyclase (AC) and increased cAMP accumulation. This holds for several classes of antidepressant drugs, and on an accelerated timescale for “rapid-acting” antidepressants like ketamine.
Using a lipid raft localized cAMP sensor, we show that Gs signaling is reduced in lipid rafts, while signaling is simultaneously promoted elsewhere in the cell. These signaling changes mirror

the changes in Gs localization observed following antidepressant treatment. Furthermore, we show that suppression of Gs signaling in lipid rafts persists at least 24 hr after cessation of antidepressant treatment.
Cellular adaptations induced by antidepressants were modeled in C6 rat glioma cells. Gs downstream signaling was analyzed following stimulation by the beta-adrenergic receptor agonist isoproterenol. cAMP levels were determined in live cells using a fluorescent cAMP sensor (Montana Molecular). This sensor was expressed either cytoplasmically, in lipid raft membranes, or in non-raft membrane regions. Gs localization was determined following membrane isolation and sequential detergent extraction, and quantified using the Wes protein quantification system (Protein Simple). High resolution cellular imaging and Fluorescence Recovery After Photobleaching (FRAP) experiments were performed on an LSM 880 confocal microscope (Zeiss).
We show that suppression of lipid raft Gs signaling persists for an extended time period after antidepressant withdrawal, while increased cytoplasmic Gs signaling reverts partially or fully upon cessation of antidepressant treatment. Translocation of Gs out of lipid rafts is also persistent. These events may reflect cellular adaptations to antidepressant treatment which contribute to antidepressant withdrawal syndromes, and may aid in the discovery of new treatments and strategies to mitigate these side effects.

Disclosures: **N.B. Senese:** None. **M.M. Rasenick:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Lundbeck. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pax Neuroscience.

Poster

236. Depression: Pathology

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 236.04/T13

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: R41 MH113398
NIH R01AT009169
VA BX001149
VA BX004475

Title: A biomarker-based high-throughput screen for antidepressant efficacy

Authors: *J. M. SCHAPPI[1,2], M. BEN AISSA[1], J. R. HICKOCK[1], M. M. RASENICK[3,2,4,5]; [1]Col. of Pharm. (UIC), Chicago, IL; [2]Pax Neurosci., Glenview, IL; [3]Dept Physiol, Biophysics, Univ. of Illinois at Chicago Col. of Med., Chicago, IL; [4]Jesse Brown VAMC, Chicago, IL; [5]Psychiatry, Univ. of Illinois at Chicago, Chicago, IL

Abstract: Development of new antidepressants has stagnated. With the recent exception of ketamine, no truly novel antidepressant compounds have been FDA-approved in the past four decades. One biosignature shared by antidepressants from diverse chemical and functional classes is their ability to alter membrane G protein signaling; specifically, to promote the movement of $G\alpha_s$ out of lipid rafts and into non-raft membrane fractions, enhanced coupling with adenylyl cyclase, and increased generation of cAMP in response to agonist. This effect occurs independent of the actions of many antidepressants on monoamine transport; and the cellular model utilized, C6 glioma, does not express monoamine reuptake transporters. We sought to develop a technology that would consider the delayed action of most antidepressant drugs (as well as the rapid action of ketamine) and employ $G\alpha_s$ -mediated enhancement of cellular cAMP generation as the basis of a high-throughput screen utilizing AlphaScreen technology. Challenges of this approach include the need to treat cells for an extended time period (3 days in C6 glioma) in a large multi-well plate format, incorporation of automation in cell and biochemical assay handling, and the exclusion of compounds acutely potentiating cellular cAMP production but lacking effect on membrane G protein disposition. Still, this may represent a low-cost, high-throughput method for identifying novel compounds with antidepressant potential.

Disclosures: **J.M. Schappi:** A. Employment/Salary (full or part-time):; University of Illinois at Chicago; Pax Neuroscience. **M. Ben Aissa:** A. Employment/Salary (full or part-time):; University of Illinois at Chicago. **J.R. Hickock:** A. Employment/Salary (full or part-time):; University of Illinois at Chicago. **M.M. Rasenick:** A. Employment/Salary (full or part-time):; University of Illinois at Chicago; USVA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pax Neuroscience.

Poster

236. Depression: Pathology

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 236.05/T14

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: NIMH MH077159

Title: Cellular identification of corticotropin-releasing hormone in the anterior cingulate cortex of human subjects

Authors: *H. OH[1], D. A. LEWIS[2], E. SIBILLE[3];
[1]CAMH, Toronto, ON, Canada; [2]Dept Psychiatry, Univ. Pittsburgh, Pittsburgh, PA; [3]CAMH - Univ. of Toronto, Toronto, ON, Canada

Abstract: *Introduction.* Corticotropin-releasing hormone (CRH) is implicated in various psychiatric illness. Our recent meta-analysis of eight microarray studies revealed significant reduction of CRH expression in corticolimbic regions of patients with major depressive disorder (MDD), suggesting that CRH-expressing cells may be affected in MDD. CRH's action and regulation are mainly studied in the context of HPA axis, however, CRH is also expressed extrahypothalamically, mostly in GABAergic neurons in mouse brain. The characteristic of CRH (+) cells in human brains and its association to disease are largely unknown. The aim of this study is to investigate the cellular identity of CRH (+) neurons in human cortex and to characterize cellular specificity of CRH reduction in brains of MDD patients.
Methods. Using fluorescent in situ hybridization (FISH), we labeled cells in the anterior cingulate cortex (ACC, Brodmann area 25) of human control subjects with probes targeting CRH and markers of excitatory (SLC17A7), inhibitory (GAD1) neurons, as well as three major types of inhibitory interneurons (PVALB, SST, VIP). Coexpression patterns of CRH and neuronal markers were analyzed. In parallel, CRH expression level was quantified using qPCR in the gray matter of ACC of MDD subjects and matched controls (n=6/group). Same FISH approach was used to investigate CRH (+) cell density changes in MDD.
Results. 27% of GAD1-expressing GABA neurons in ACC was labeled with CRH. ~80% of CRH (+) cells were GABAergic and found across layers I-VI, 10% was glutamatergic and concentrated in deep layers. CRH (+) GABA neurons coexpressed markers of subpopulation of interneurons: VIP (52%), SST (7%), PVALB (7%). Notably 34% of CRH (+) cells expressed GAD1, but none of three GABA interneuron markers. MDD patients had reduced CRH expression in the ACC compared to control (-24.4%, p=0.018), however, there was no significant group difference in the CRH (+) cell density (-4.0%, p=0.35).
Conclusions. In the human ACC, CRH is mostly expressed in GABA neurons and overlapping with major classes of GABA interneurons. Yet, a substantial fraction of CRH (+) GABA cells does not contain detectable levels of markers of major cortical interneurons. CRH expression is significantly reduced in the brains of MDD patients, without changes in cell density, raising the question as to which subgroup of CRH (+) neurons is affected in MDD.

Disclosures: H. Oh: None. **D.A. Lewis:** None. **E. Sibille:** None.

Poster

236. Depression: Pathology

Location: Hall A

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Program #/Poster #: 236.06/T15

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: Canadian Institutes of Health Research
Fonds de la Recherche en Santé du Québec
Healthy Brains for Healthy Lives

Title: snRNA-seq in the post-mortem prefrontal cortex implicates OPCs and deep layer excitatory neurons in major depressive disorder

Authors: *M. MAITRA[1], C. NAGY[1], A. TANTI[1], M. SUDERMAN[2], J. F. THÉROUX[1], M. A. DAVOLI[1], K. PERLMAN[1], V. YERKO[1], S. TRIPATHY[3], P. PAVLIDIS[3], N. MECHAWAR[1], J. RAGOUSSIS[4], G. TURECKI[1];
[1]McGill Group for Suicide Studies, Douglas Mental Hlth. Univ. Inst., Montreal, QC, Canada; [2]Population Hlth. Sci., Bristol Med. Sch., Bristol, United Kingdom; [3]Psychiatry, Univ. of British Columbia, Vancouver, BC, Canada; [4]McGill Univ. and Genome Quebec Innovation Ctr., Montreal, QC, Canada

Abstract: Background: Measuring transcriptomic and epigenetic changes in homogenized brain tissue can mask cell-type specific effects due to heterogeneity in cell-type composition. This is especially relevant in complex disorders, such as depression, in which a variety of cell-types and biological processes have been implicated - including altered monoaminergic signaling, altered synaptic transmission, inflammation, and blood-brain barrier impairment. We used high-throughput single-nucleus RNA-sequencing (snRNA-seq) to genetically define cellular subgroups in the brain and to identify cell-type specific differentially expressed genes (DEGs) in major depressive disorder (MDD).
Methods: We applied commercially available droplet-based high-throughput snRNA-seq technology from 10X Genomics to characterize single-nucleus transcriptomic profiles of almost 80,000 individual nuclei from the dorsolateral prefrontal cortex of 34 male subjects - 17 depressed patients who died by suicide and 17 psychiatrically healthy controls. The Seurat and lme4 R packages was used for data analysis. Fluorescent *in situ* hybridization (FISH) was performed for validating findings.
Results: Unsupervised graph-based clustering for identifying cell-types was followed by cell-type annotation using known marker genes. We identified 10 excitatory neuronal clusters, 7 inhibitory neuronal clusters, and 9 glial clusters - including astrocytic, oligodendrocytic, and oligodendrocyte precursor clusters. Differential gene expression analysis within each cell-type cluster revealed 16 significantly upregulated and 80 significantly downregulated genes in 16 out of the 26 cell-types identified (FDR < 0.10). An OPC cluster (OPC2) and a deep layer excitatory neuronal cluster (Ex7) contributed 45% of the differentially expressed genes although they accounted for only 5.5% of the nuclei analyzed, thus indicating the potential importance of these cell-types in MDD. Selected targets were validated with FISH.
Conclusion: DEGs were enriched for genes related to synaptic function, immune processes, and, cytoskeletal and kinesin proteins. Several of these genes have previously been implicated in depression, and this study elucidates further their potentially cell-type specific roles in MDD pathology. To complement our transcriptomic results, our future work will measure cell-type specific DNA methylation changes in broad categories of cell-types separated by fluorescence assisted nuclei sorting. Multiomic analysis of the combined dataset should provide further insight into the molecular mechanisms of MDD.

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Poster

236. Depression: Pathology

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 236.07/T16

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: RQSHA Pilot Project Funding

Title: Profiling brain-derived exosomes in the prefrontal-cortex of individuals with major depressive disorder

Authors: ***P. IBRAHIM**[1,3], C. NAGY[3], J.-F. THEROUX[3], G. TURECKI[3,2];
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Abstract: Background: Major Depressive Disorder (MDD) is one of the leading causes of disability worldwide, affecting 20% of the population. With our limited understanding of processes underlying the depressive state in the brain, the magnitude of this problem will continue to grow. The environment has been thought to play a role in the disease development, resulting in biological changes mediated by epigenetic mechanisms. MicroRNA's (miRNA) are well known epigenetic regulators that have recently been found to be packaged into exosomes, which are small extracellular vesicles of endosomal origin. Exosomes have emerged as means of intercellular communication, a process that is also disrupted in the brain in the depressed state. They are thought to transfer miRNA between cells, and this can alter gene expression in recipient cells. Therefore, we hypothesize that exosomal cargo is altered in MDD patients compared to healthy controls. Our aim is to extract exosomes from human post-mortem prefrontal cortex, a region previously associated with depression, and profile the exosomal cargo. We will then attempt to elucidate miRNA's that are differentially expressed between MDD patients and healthy controls.
Methods: Post-mortem brain tissue from the prefrontal-cortex was mildly dissociated in the presence of collagenase type III. Residual tissue, cells, and large vesicles were removed, and exosomes were extracted using size exclusion chromatography. The quality was assessed by western blots, probing for Calnexin, BiP, VDAC, TSG101, and CD9, and by transmission electron microscopy (TEM). A small RNA library was constructed and sequenced using the Illumina Platform to assess the quality as well.
Results: Western blots confirmed the presence of endosomal and exosomal markers (TSG101, CD9), respectively, and little to no ER (Calnexin), Golgi (BiP), or mitochondria (VDAC)

contamination. TEM images showed typical cup-shaped morphology within the expected size range (30-150 nm). Sequencing results revealed miRNA and mRNA relevant to both exosomes and brain function.
Conclusions: High quality exosome extractions can be obtained from post-mortem brain tissue using our method. We will proceed with extractions from 20 MDD patients and 20 psychiatrically healthy controls to profile the exosomal content and compare between groups. This will be the first study to profile brain-derived exosomes in the context of depression. This will provide novel mechanistic insights into the pathophysiology of MDD and will serve as a starting point to examine the potential role of exosomes in MDD pathology.

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Poster

236. Depression: Pathology

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Program #/Poster #: 236.08/T17

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: CIHR project grant BSB

Title: Oligodendrocyte lineage cells and myelination in the human uncinate fasciculus of adults with a history of child abuse

Authors: *K. PERLMAN, J. KIM, A. TANTI, D. ALMEIDA, M.-A. DAVOLI, D. MIRAULT, N. MECHAWAR;
Douglas Mental Hlth. Univ. Inst., Montreal, QC, Canada

Abstract: Child abuse (CA), defined as childhood sexual, physical or emotional abuse or neglect by a parent or caregiver, causes lasting pain and hardship for countless individuals around the world. CA strongly increases the lifetime risk of suffering from major depression and predicts an unfavorable course for the illness as well as poorer treatment outcomes. Recently, our group reported a strong association between CA and oligodendrocyte (OL) dysfunction, as well as myelin disruptions in cortical white matter. The uncinate fasciculus (UF), which connects the temporal and frontal lobes, is a major long-range association tract that is particularly relevant to CA and mood disorders. Therefore, we set out to examine the cell morphology, cell density and expression of myelin-related genes and proteins in the UF from depressed suicides having suffered from severe CA (DS-CA) compared to matched depressed suicides without CA (DS) and healthy controls (CTRL). Immunoblotting was used to quantify the expression of myelin-constituent proteins and quantitative RT-PCR was used quantify expression of myelin-constituent genes. Furthermore, immunohistochemistry and stereology were used to investigate OL-lineage cell density and morphology. No significant between-group differences were

observed in either the gene or protein expression of PLP, MAG, MOG, MBP, or MOBP. Additionally, the density and cell body volumes of Nogo-A+ (mature OLs) and PDGFRa+ (OL progenitors) cells were not significantly different between groups. These results, indicating that there are no CA-specific alterations for these metrics, will be presented together with those generated by ongoing full transcriptome RNA sequencing. Combined, these convergent analyses should reveal either that the UF is resilient to the effects of CA, or that it is affected differently than cortical white matter.

Disclosures: K. Perlman: None. **J. Kim:** None. **A. Tanti:** None. **D. Almeida:** None. **M. Davoli:** None. **D. Mirault:** None. **N. Mechawar:** None.

Poster

236. Depression: Pathology

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 236.09/T18

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Title: Small nucleolar RNAs in major depression and antidepressant response

Authors: *R. LIN[1], J. LOPEZ[1], L. FIORI[1], J.-F. THEROUX[1], Z. AOUABED[1], H. PENG[1], C. ERNST[1], E. IBRAHIM[2], C. CRUCEANU[1], R. BELZEAUX[1], C. AN-BIND WORKING GROUP[3], J. A. FOSTER[4], S. KENNEDY[5], G. TURECKI[1];
[1]McGill Group for Suicide Studies, Douglas Mental Hlth. Univ. Institute, McGill Univ., Montreal, QC, Canada; [2]Aix Marseille Univ, CNRS, INT, Inst. Neurosci Timone UMR 7289, Marseille, France; [3]The Canadian Biomarker Integration Network in Depression (www.canbind.ca), Toronto, ON, Canada; [4]Psychiatry & Behav Neurosci, McMaster Univ., Hamilton, ON, Canada; [5]Dept. of Psychiatry, Univ. Hlth. Network, Univ. of Toronto, Toronto, ON, Canada

Abstract: Background:
Major depressive disorder (MDD) is a prevalent global disorder treated primarily by antidepressants (AD). However, 30%-40% of subjects fail to respond after multiple attempts. Thus, discovering biomarkers for AD-response would greatly improve treatment outcome. Small non-coding RNAs (sncRNAs) have recently been identified as feasible biomarkers associated with disease states, as they are stably circulated in peripheral blood and are involved in several post-transcriptional modification processes. Here I profile small nucleolar RNAs (snoRNAs), since they have been disregarded in MDD pathology, leaving room for novel discoveries. To holistically evaluate snoRNAs as candidate biomarkers, I am using a unique combinatorial approach of profiling snoRNAs expression in both post-mortem human brain tissue and living human peripheral blood.
Methods:

Post-mortem brain tissue was collected from individuals who died by suicide during an MDD episode, and psychiatrically normal individuals who died suddenly. Peripheral blood was collected from two independent cohorts consisting of MDD subjects treated with AD for 8 weeks. Subjects were assessed for MDD severity using the Montgomery-Asberg Depression Rating Scale (MADRS) before treatment (Week 0) and afterwards (Week 8). Post-AD treatment subjects were separated into responders (≥50% decrease in MADRS score) or non-responders. Small RNA-sequencing was used to generate whole sncRNA-transcriptome expression data from post-mortem brain and peripheral blood.

Results:

SNORD90 was differentially expressed between MDD-suicides and controls in post-mortem brain and specifically upregulated in AD-responders across time. All sequencing results have been validated via qPCR. Using in-silico analysis, Neuregulin 3 (NRG3) has been identified as a potential target of SNORD90. Preliminary results suggest SNORD90 is negatively regulating NRG3 expression.

Conclusions:

To my knowledge, this is the first study focusing on snoRNAs in the context of MDD and AD-response. This noval information will further our understanding of MDD pathology, and has potential clinical impact as it also shows characteristics of a biomarker.

Disclosures: R. Lin: None. **J. Lopez:** None. **L. Fiori:** None. **J. Theroux:** None. **Z. Aouabed:** None. **H. Peng:** None. **C. Ernst:** None. **E. Ibrahim:** None. **C. Cruceanu:** None. **R. Belzeaux:** None. **C. AN-BIND working group:** None. **J.A. Foster:** None. **S. Kennedy:** None. **G. Turecki:** None.

Poster

236. Depression: Pathology

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Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 236.10/T19

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: NIMH 094445

Title: Characterizing signal transduction networks in postmortem depressed-suicide subjects

Authors: *S. M. O'DONOVAN[1], E. BENTEA[2], K. ALGANEM[1], R. E. MCCULLUMSMITH[1]; [1]Univ. of Toledo, Toledo, OH; [2]Dept. of Psychiatry, Univ. of Cincinnati, Cincinnati, OH

Abstract: Suicide is one of the top 20 causes of death worldwide, but few interventions consistently reduce suicidal thoughts and behaviors. Our limited understanding of the neurobiology of suicide hinders development of efficacious and safe interventions. Kinases fine-tune signaling in complex biological networks. Kinase gene and protein expression levels are

reduced in depressed subjects who die by suicide. Measuring individual kinase expression alone is not sufficient to understand the kinome, the complex network of kinase interactions which represents the intrinsic state of kinases. The kinome has not been studied in subjects who died by suicide. We investigated changes in serine/threonine kinase activity in the frontal cortex of depressed subjects who died by suicide and comparison subjects (n=10 per group, pooled) using a kinome peptide array-based system (PamGene12). We demonstrate large scale abnormalities in activity (log2 fold change >0.15 or <-0.15) of kinases in depressed subjects who die by suicide. Upstream kinase analysis identified kinases including ERK, protein kinase A (PKA) and dual-specificity tyrosine phosphorylation-regulated kinase (DYRK) with a z-score +/-3. The kinase data undergo connectivity mapping using the Library of Integrated Network-based Cellular Signatures (LINCS) database. Connectivity mapping links changes in patterns of gene expression induced by altered kinase network activity in depressed-suicide subjects, with chemical perturbagens that induce similar and opposing patterns of change in gene expression. Kinases have the potential to modulate complex behaviors like suicide but are yet to be exploited therapeutically. Studying the kinome will increase our understanding of the neurobiology of suicide and may lead to the development of novel interventions for suicide.

Disclosures: S.M. O'Donovan: None. **E. Bentea:** None. **K. Alganem:** None. **R.E. McCullumsmith:** None.

Poster

236. Depression: Pathology

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Program #/Poster #: 236.11/T20

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: American Foundation for Suicide Prevention
Loyola University Chicago

Title: Epitranscriptomic modifications of the 5-HT_{2C} receptor predict completed suicide

Authors: *K. LOTESTO, M. SODHI;
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Abstract: Suicide is a preventable tragedy with more than 40,000 deaths per year in the United States. Accumulating data indicate that an epitranscriptomic process known as ‘RNA editing’ may contribute to the pathophysiology of suicide. RNA editing is an epitranscriptomic process that creates sequence changes in RNA, most commonly catalyzed by ADAR enzymes (ADARs1-3) in the mammalian brain. Seven previous studies indicate increased 5-HT_{2C} receptor (5-HT_{2C}R) RNA editing in the prefrontal cortex (PFC) in cases of suicide. We now present data from analyses of 5-HT_{2C} RNA editing in a much larger cohort of postmortem subjects (n=114) to

attempt to consolidate previous findings. We tested RNA from the gray matter of the PFC (BA46) of 80 postmortem subjects with major depressive disorder who either died by suicide (MDD-S) or other causes of death (MDD-NS) in addition to 34 control subjects (CTRLs). We measured gene expression using quantitative polymerase chain reaction (QPCR) and we measured RNA editing using tagged next generation sequencing. We report increased levels of 5-HT2C RNA editing in the MDD-S group ($p<0.05$) and increased levels of RNA editing at the 5-HT2C 'A' site ($p<0.05$). Meta-analysis including 4 similar studies of PFC revealed that increased 5-HT2C RNA editing at the 'A' site is associated with completed suicide ($p<0.001$). In summary, we report increased 5-HT2C RNA editing in the frontal cortex in cases of suicide. Increased 5-HT2C RNA editing leads to reduced 5-HT2CR signal transduction. In the PFC, 5-HT2CRs are expressed in GABA interneurons, so reduced 5-HT2CR activity may lead to disinhibition of glutamatergic pyramidal neurons of the PFC. Therefore RNA editing may contribute to the pathophysiological pathways leading to suicide. These data may facilitate the development of improved antidepressant drugs in addition to identifying a potential biomarker of completed suicide. *Acknowledgments:* The authors thank Joel E. Kleinman and Barbara Lipska for postmortem tissue included in this study. Funded by the American Foundation for Suicide Prevention and Loyola University.

Disclosures: K. Lotesto: None. **M. Sodhi:** None.

Poster

236. Depression: Pathology

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 236.12/U1

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: JSPS KAKENHI(16K10189)
"Integrated Research on Neuropsychiatric Disorders" conducted under the Strategic Research Program for Brain Sciences from the MEXT and AMED
Core Research for Evolutional Science and Technology (CREST)
Industrial Strategic Research and Development from Yamaguchi Prefecture
Pfizer

Title: DNA methylation markers of early onset major depressive disorder disorder

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Abstract: Background: Major depressive disorder (MDD) is a well-known heterogeneous disease, and multiple factors, including genetic and environmental ones, contribute to its pathophysiology. Recently, we reported that the gene expression profile of leukocytes in early-onset depression (EOD; onset age < 50) was different from that in late-onset depression (LOD; onset age ≥50). We postulate that DNA methylation has a greater influence on EOD than on LOD. The purpose of this study was to identify methylation markers that are specific to EOD patients.
Method: This study was approved by the Institutional Review Board of Yamaguchi University Hospital, and informed consent was obtained from all subjects. We performed genome-wide DNA methylation analysis of leukocytes of patients with EOD (n=10) and LOD (n=25), and compared the results to those of healthy control subjects (HC; n=30).
Results and discussion: We detected several methylation markers that could differentiate EOD from HC with high accuracy. Some of the identified EOD methylation markers were validated by pyrosequencing and bisulfite sequencing. They could be used to differentiate EOD from HC using independent samples. The alternation of methylation of DNA from EOD was also different from that from LOD. The spread of the EOD's distribution is smaller than those of HC and LOD. These results suggest that in the identification of methylation markers of MDD, the onset age should be included as a critical factor.

Disclosures: H. Yamagata: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Pfizer, Eisai, MSD. **H. Ogihara:** None. **K. Matsuo:** None. **S. Uchida:** None. **T. Seki:** None. **M. Kobayashi:** None. **K. Harada:** None. **C. Chen:** None. **S. Miyata:** None. **M. Fukuda:** None. **M. Mikuni:** None. **Y. Watanabe:** None. **S. Nakagawa:** None.

Poster

236. Depression: Pathology

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 236.13/U2

Topic: G.07. Other Psychiatric Disorders

Title: Increased cell number with reduced nitric oxide levelin the anterior-pituitary region of suicide completers

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Abstract: Suicidal behavior is a complex human behavior and current data suggests that suicide is an increasing cause of death among young people. The neurobiology of suicide is unknown and data investigating the role of the pituitary in suicidal behavior is scarce. Imaging data suggests that this gland increases in size in patients with major depression and recent data implicates hyperactivity of the hypothalamus-pituitary-adrenal axis in suicidal behavior. In this study, we evaluate the size and number of cells as well as markers related to oxidative stress and lipid peroxidation of the anterior and posterior sections of the pituitary gland of male suicide completers. Stereological analysis is used to quantify the total cell number in anterior- and posterior-pituitary regions. We examined nitric oxide (NO) levels, Zinc (Zn) levels, superoxide dismutase (SOD) activity, 4-hydroxy-alkenals (4-HDA), malondialdehyde (MDA) and metallothioneins (MTs). Our results indicate that the anterior-pituitary region of suicide completers exhibits increased weight, likely due to an enhanced number of cells compared to the control group. In addition, we found a reduction of NO levels with higher SOD activity in the anteriorpituitary region of suicide victims. No changes in Zn, MDA, MTs, 4-HDA or MDA were observed in tissue of suicide completers compared to the control group. This study demonstrates that there is an increased number of cells, with an imbalance in oxidative stress without a process of lipid peroxidation in the anterior-pituitary region of young male suicide completers.

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Poster

237. Genetic and Molecular Mechanisms Underlying Alcohol Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 237.01/U3

Topic: G.08. Drugs of Abuse and Addiction

Support: PUT1686

Title: The effect of DNA methylation and demethylation in a rat model of alcohol use disorder

Authors: *K. NIINEP[1], K. ANIER[1], T. ETELÄINEN[2], P. PIEPPONEN[2], A. KALDA[1];
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Abstract: The alcohol use disorder (AUD) is complex psychiatric disorder that is characterized by excessive alcohol drinking, alcohol dependence and relapses even after long periods of abstinence. It is a devastating public health problem in which both genetic and environmental factors play a role. It is well known that alcohol use disorder is highly inheritable. The fact that alcoholism tends to run in families has been known for a long time and almost all twin studies have established that in alcoholism there is a genetic factor and heritability ranges from 40-70% (Kendler *et al.*, 2012). Furthermore, there are growing evidence that epigenetic mechanisms, such as histone modifications and DNA methylation/demethylation are involved in the development of AUD (Al Ameri *et al.*, 2014; Simon-O'Brien *et al.*, 2015). However, there are only few studies that are focused on DNA modifications and gene expression changes in AUD. Our aim for this study was to investigate whether alcohol-induced changes in gene expression and enzyme activity are influenced by epigenetic modifying enzymes called DNA methyltransferases (enzymes that catalyze the process of DNA methylation; *Dnmt1, Dnmt3a, Dnmt3b*) and demethylases (ten-eleven translocases enzymes that are responsible for DNA demthylation; *Tet1-3*) using animal models of alcohol drinking. We hypothesize that the long-term gene expression changes in the brain, via the epigenetic modification, is underlying molecular mechanism of alcohol drinking induced AUD and relapses after abstinence.
We used rat model of AUD, where there were three groups of animals: alcohol-preferring (AA-ethanol, Alko Alcohol rats with access to alcohol), AA-water (AA rats with access only to water) and Wistar-water (rats who have had only water). The AA-ethanol rats had access to alcohol (10% ethanol solution) for 90 min every other day for three weeks. Our initial qPCR results in the *nucleus accumbens* (NAc) show, that voluntary alcohol drinking induces epigenetic modifications in the AA-ethanol rats, where *Dnmt1, Dnmt3a, Dnmt3b* were overexpressed, while *Tet* family transcripts where slightly downregulated compared to AA-water and Wistar-water rats. To support our qPCR results we used ELISA-like method for DNMT/TET activity analysis and found that DNMT activity had increased in AA-ethanol group compared to AA-water rats while we did not find statistically significant difference in TET activity.
This data indicate that alcohol drinking may disturb the equilibrium between DNA methylation and demethylation processes in the NAc and cause changes in gene expression.

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Poster

237. Genetic and Molecular Mechanisms Underlying Alcohol Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 237.02/U4

Topic: G.08. Drugs of Abuse and Addiction

Support: R25N S080687-06
P20 GM103642

Title: Tip60 as a significant player in the creation of alcohol tolerance

Authors: *L. RAMOS-RODRIGUEZ, C. BILLINI-GUZMAN, E. M. FIGUEROA-PAGAN, J. L. AGOSTO-RIVERA, A. GHEZZI;
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Abstract: Alcohol consumption has many effects in the human body, including the development of neural adaptations that lead to tolerance and dependence. In Drosophila melanogaster these adaptations also take place. In particular, alcohol tolerance is known to be caused by a set of adaptations that occur in the brain once the organism has been exposed to the drug. This homeostatic response has been shown to involve upregulation of specific genes in the brain to make it less susceptible to alcohol sedation. Because of its powerful genetic toolkit, Drosophila is uniquely positioned to decipher the genetic components of these adaptations. Now, we are looking at the epigenetic processes that alter chromatin structure and gene expression that lead to the development of tolerance. It has been shown that acetylation of H3 and H4 is associated with alcohol exposure and the development of tolerance. Our main goal is to evaluate which of the known histone acetyltransferases (HATs) are recruited in the creation of alcohol tolerance and to tie the specific HATs to expression of genes that are associated with alcohol tolerance. Using the UAS-Gal4 system, we knock-down gene expression of the histone acetyltransferase Tip60 in the whole fly brain and determine its effect on alcohol tolerance. Tolerance is measured as a change in the rate of sedation between exposures as quantified by a Drosophila Activity Monitor. We found that flies with Tip60 gene KD did not have a significant increase in time for complete sedation between exposure, compared with the control group. This suggests that the Tip60 is necessary for the proper development of tolerance to alcohol.

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Poster

237. Genetic and Molecular Mechanisms Underlying Alcohol Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 237.03/U5

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant Y1AA-3009

Title: Cellular aging in alcohol use disorder

Authors: *L. M. CARVALHO[1], C. WIERS[1], P. MANZA[1], H. SUN[1], M. SCHWANDT[1], G.-J. WANG[1], R. GRASSI-OLIVEIRA[2], A. BRUNIALTI GODARD[3], N. VOLKOW[1]; [1]NIAAA, Bethesda, MD; [2]Brain Inst., Pontifical Catholic Univ. of Rio Grande do Sul, Rio Grande do Sul, Brazil; [3]Federal Univ. of Minas Gerais, Belo Horizonte, Brazil

Abstract: Human telomeres are tandem repeats at the end of chromosomes which protect DNA from degradation. Telomeres are not fully replicated during every cell division and become progressively shorter over the lifespan. In addition, life stressors, drug use, obesity and psychiatric disorders could accelerate cell aging and affect telomere length (TL). Though, studies have evaluated the effects of alcohol consumption on TL, the results have been inconsistent, which might reflect the diverse drinking cut-offs and categorizations and the interaction of alcohol misuse with life stressors. To help clarify the effects of excessive alcohol consumption and TL, here we assess the association of TL with alcohol use disorder (AUD), drinking behaviors, lifetime stress and age. TL was quantified as the telomere to albumin ratio (T/S ratio) obtained from peripheral blood DNA using the monochrome multiplex quantitative PCR assay, from 260 participants with AUD and 449 non-dependent healthy controls (HC) from an existing National Institute on Alcohol Abuse and Alcoholism (NIAAA) database. AUD participants showed shorter TL compared to HC with both, age (β= -.277, p<.0001) and AUD (β = -.424, p<.0001), as independent predictors, as well as a significant AUD with age (β= -.322, p=.002) interaction effect on TL. In AUD participants, TL was associated with cognitive instability (r = 0.210, p<0.001) and motor impulsiveness (r = 0.135, p<0.05). Alcohol drinking behavior (heavy drinking days, drinks per weeks, heavy drinking years, total lifetime drinking in grams) were not associated with TL (p>.05). The failure to see an association between TL and alcohol drinking behaviors despite an association with AUD could reflect the large variability in the rate of alcohol metabolism and bioavailability between subjects. In AUD we did not observe an association between TL and childhood trauma (childhood trauma questionnaire and early life stress questionnaire) (p>0.5); whereas we did not have sufficient power to assess it in HC since only 10 reported a history of childhood trauma. Failure to observe a stress effect on TL in AUD could reflect the relatively large effect of AUD and age on TL that might have interfered with our ability to detect it. Our results show both an effect of AUD on TL that is independent of age as well as a significant AUD by age interaction on TL. These findings are consistent with accelerated cellular aging in AUD.

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Poster

237. Genetic and Molecular Mechanisms Underlying Alcohol Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 237.04/U6

Topic: G.08. Drugs of Abuse and Addiction

Support: Plan Nacional sobre Drogas Grant 2016I025

Title: Clusterin gene polymorphisms associated to drug abuse

Authors: I. PALLARDO[1], J. R. MUÑOZ-RODRÍGUEZ[2], C. GONZALEZ-MARTIN[1], ***L. F. ALGUACIL**[1];
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Abstract: Clusterin is a multifunctional protein that was found elevated in the plasma of patients with obesity and poor control over eating, and was therefore proposed to be a potential biomarker of food addiction (Rodríguez-Rivera et al., World J Surgery 2019; 43:744-750). A recent study further showed that the levels of clusterin in saliva from smokers were dependent on the history of tobacco use the and the daily number of cigarettes consumed; moreover, these levels significantly decreased 6 months after smoking cessation (Pallardo et al., Tob Prev Cessation 2018;4 Suppl:A160). These studies together with other experimental work conducted in animal models strongly suggest that clusterin could be functionally involved in different kind of addictions. In the present work we have checked 44 selected single nucleotide polymorphisms (SNP) of the clusterin gene in 500 cocaine/alcohol abusers (18-75 years of age, 64% males) and 500 controls. DNA samples were provided by the BioBank RTA, integrated in the Valencian Biobanking Network, and the Spanish DNA National Bank Carlos III. Genetic analysis was performed in the Spanish National Center for Genotyping (CeGen-ISCIII) by using a multiplexing assay (Sequenom iPLEX Gold). Samples were processed following standard operating procedures with the appropriate approval of the Ethical and Scientific Committees. Logistic regression was used to study associations between SNPs and drug addiction. According to odds ratios and Wald p-values, seven SNPs were found to be significantly associated with drug abuse: of particular interest were rs867231 and rs867232 as well as rs11787077, since they were also found to be associated with the use of tobacco and alcohol among controls and with the addiction scores among addicts. The results obtained extends previous work supporting the idea that clusterin could play a role in addiction where it might serve as a potential diagnostic / prognostic biomarker.

Disclosures: L.F. Alguacil: None. **I. Pallardo:** None. **J.R. Muñoz-Rodríguez:** None. **C. Gonzalez-martin:** None.

Poster

237. Genetic and Molecular Mechanisms Underlying Alcohol Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 237.05/U7

Topic: G.08. Drugs of Abuse and Addiction

Support: NIAAA Grant AA025721
NINDS Grant NS091144
NIAAA Grant F31AA027432
NSF GRFP 2016217742

Title: Ethanol exerts its influence on dopaminergic GABA co-release via its metabolite acetaldehyde in the striatum

Authors: *K. KAGANOVSKY[1], S. GANESAN[1], J.-I. KIM[1], S. WANG[2], L. CHEN[3], J. B. DING[1];
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Abstract: It is understood that repeated binge-like drinking leads to alcoholism; however, the mechanisms underlying the rewarding effects of alcohol are less clear. Alterations in the dopaminergic system are particularly relevant to alcohol reward because dopamine (DA) is critical for both natural and drug reward. It is now known that, in addition to DA, DA neurons can co-release fast-acting neurotransmitters including glutamate and GABA. We previously showed that GABA co-released from midbrain DA neurons is mainly synthesized by the non-canonical GABA synthesizing enzyme, aldehyde dehydrogenase 1a1 (ALDH1A1). Interestingly, we also found that ethanol could attenuate GABA co-transmission in the striatum; however, the mechanism remains unknown. Here, we demonstrate that ethanol does not directly inhibit the oxidation of ɣ-aminobutyraldehyde (ABAL, a precursor molecule for GABA synthesis) by ALDH1A1. Instead, we found that an ethanol metabolite, acetaldehyde, can inhibit ALDH1A1 and its synthesis of GABA. In addition, direct treatment of acetaldehyde can mimic ethanol action, resulting in a similar decrease in GABA co-release recorded from striatal SPN. Biochemical assays further confirm that acetaldehyde works as a potent competitor and inhibits ABAL oxidation, a key step for GABA synthesis mediated by ALDH1A1. These findings suggest that ethanol may exert its influence on dopaminergic GABA co-release via its metabolites. Together with our previous results showing that a reduction in GABA co-release is associated with increased alcohol preference, these data show that acetaldehyde may mediate the relationship between alcohol, GABA co-release, and reward.

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Poster

237. Genetic and Molecular Mechanisms Underlying Alcohol Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 237.06/U8

Topic: G.08. Drugs of Abuse and Addiction

Title: Prevention of glutamate excitotoxicity in lateral habenula alleviates ethanol withdrawal induced somatic and behavioral effects in ethanol dependent mice

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Abstract: Lateral habenula (LHb) an epithalamic nuclei is a highway to the midbrain for processing aversion and reward. Neurodegeneration of Fasciculus Retroflexus (FR) pathway (glutamatergic projection from LHb to RMTg) have been suggested in cocaine, morphine and nicotine addiction but remains unexplored in ethanol addiction. Progressive increase in ethanol consumption leads to alterations in brain structures which reduce behavioral control promoting further ethanol abuse and neurodegeneration. We hypothesize that chronic ethanol induced glutamate excitotoxicity plays an important role in neurodegenerative changes lead progression of dependence or withdrawal effects. Therefore, we investigated if glutamate excitotoxicity reduction in LHb influences ethanol dependence and withdrawal in mice. Adult male Swiss Albino mice (25-30g) were cannulated into LHb. Mice were made dependent using ethanol (4g/kg; 20% w/v; intragastric) for 10 days. Different groups of ethanol dependent mice were chronically treated intra-LHb with PDC (glutamate transporter inhibitor) or RO25-6981 (NR2B inhibitor) or BAPTA-AM (calcium chelator) and tested for somatic signs and behavioral effects in open field test, elevated plus maze and light and dark box. Further, the brains of these animals were processed for histological staining. We found that ethanol (4g/kg; 20% w/v) administration for 10 days significantly produced withdrawal symptoms in ethanol dependent mice. Further, administration of PDC increased ethanol dependence and aggravated the withdrawal symptoms. In contrast, administration of BAPTA-AM and RO25-6981 significantly decreased the somatic signs, locomotor activity as well as anxiogenic effects in ethanol withdrawn mice. Moreover, in histological staining, we observed that while PDC (increased synaptic glutamate level) increased the cell damage at LHb, BAPTA-AM and RO25-6981 showed the neuroprotection as compared to ethanol dependent mice. Thus, the present study showed that the chronic ethanol exposure lead to the degenerative changes in LHb due to excitotoxic glutamatergic transmission and disruption of LHb increases ethanol dependence and addiction. In addition, BAPTA-AM and RO25-6981 reduced glutamate excitotoxicity within LHb consequently preventing predisposition of animals to ethanol addiction.

Disclosures: S.G. Gakare: None. **S.S. Varghese:** None. **R.R. Ugale:** None.

Poster

237. Genetic and Molecular Mechanisms Underlying Alcohol Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 237.07/U9

Topic: G.08. Drugs of Abuse and Addiction

Support: F30AA025535
P50AA022537

Title: Ndrg1 and myelin-related disease: Alcoholism, peripheral neuropathy, and sensitivity to CIPN

Authors: *G. HARRIS[1,2], W. TOMA[1], D. HUEY[3], A. D. VAN DER VAART[1], M. N. DRIVER[3], M. DAMAJ[1], M. F. MILES[1,2];
[1]Pharmacol. and Toxicology, [2]Alcohol Res. Ctr., [3]Virginia Commonwealth Univ., Richmond, VA

Abstract: Our laboratory has implicated N-myc down-regulated gene 1 (*Ndrg1*) as a potential candidate gene that modulates ethanol-induced changes in myelin-related gene expression and acute sensitivity to ethanol, as measured by loss of righting reflex (LORR) duration. Analysis of mPFC expression data found that *Ndrg1* expression was positively correlated with voluntary ethanol intake across the BXD panel of mice and demonstrated that the basal levels of *Ndrg1* mRNA expression in the mPFC across seven different strains of mice was inversely correlated with LORR duration time. We set out to determine the relationship between regulation of *Ndrg1* expression and ethanol-related behaviors. We found that mPFC *Ndrg1* expression was upregulated following 5 weeks of intermittent ethanol access in C57BL/6J (B6J) mice. We also observed an induction of *Ndrg1* in the mPFC of female B6J mice following acute ethanol exposure. Additional studies to quantify protein and protein phosphorylation states are currently underway. After observing the regulation of *Ndrg1* in wild type mice, studies were performed in a mouse line that produces a myelinating cell-selective conditional knockout (KO) of *Ndrg1*, by inducing Cre Recombinase with tamoxifen treatment. We found altered ethanol concentration preferences and total ethanol preference between the KO and control groups in a 5-week drinking study. Additionally, the KO animals also had significantly shorter onset times in the Loss of Righting Reflex (LORR) test. The alterations in ethanol concentration preference and LORR onset suggest that expression of *Ndrg1* in myelinating cells may play a role in modulating acute, initial sensitivity to ethanol. Mutations in *Ndrg1* result in a polyneuropathy: Charcot-Marie-Tooth Disease 4D. Given that mutations in *Ndrg1* are known to cause peripheral neuropathies in multiple species, we decided to characterize the peripheral nerve dysfunction that develops from conditional deletion of the gene in adult aged mice. Additionally, some evidence in clinical literature has suggested that nerve tissue levels of NDRG1 inversely correlates with chemotherapy-induced peripheral neuropathy (CIPN) following treatment with paclitaxel for breast cancer. In mice, knockout of NDRG1 in all myelinating cells produces a long-lasting hypersensitivity to touch, followed by progressive hind leg weakness. Furthermore, low dose Paclitaxel accelerates the development of neuropathy symptoms in mice that have had *Ndrg1* knocked out. This provides some evidence that levels of NDRG1 expression in nerve tissue are predictive of severity of chemotherapy-induced peripheral neuropathy following Paclitaxel treatment.

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Poster

237. Genetic and Molecular Mechanisms Underlying Alcohol Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 237.08/U10

Topic: G.08. Drugs of Abuse and Addiction

Support: F30-AA026497 (NIAAA)
P50-AA022537 (NIAAA)

Title: Deletion of chloride intracellular channel 4 (Clic4) alters voluntary ethanol consumption in mice

Authors: *R. M. WESTON[1,2], D. J. HUEY[1,2], M. N. DRIVER[1,2], G. M. HARRIS[1,2], A. VAN DER VAART[1,2], M. F. MILES[1,2];
[1]Pharmacol. & Toxicology, [2]Alcohol Res. Ctr., Virginia Commonwealth Univ., Richmond, VA

Abstract: CLIC4 is an evolutionarily conserved protein tied to diverse molecular processes including ion channel activity, actin network remodeling, intracellular trafficking, and redox reactions. *Clic4* mRNA expression is downregulated in forebrain of postmortem alcoholics and induced by acute ethanol in prefrontal cortex (PFC) of male DBA/2J (D2) mice, where its expression correlates with myelin genes. It has also been shown that overexpression of *Clic4* in PFC decreases ethanol sensitivity in D2 mice. These prior studies identify *Clic4* as an ethanol-responsive gene also capable of modulating ethanol-related behavior. In the present body of work, we further characterized cellular expression of brain CLIC4 and its regulation of ethanol behaviors using C57BL/6J (B6) mice. Immunofluorescent labeling of B6 mouse prefrontal cortex sections revealed high expression of CLIC4 protein in CC1+ and CNPase+ oligodendrocytes including within nuclear, cytosolic, and myelin subcellular compartments. CLIC4 was also found to be expressed to a lower degree in the cytosol of CaMKIIα+ and NeuN+ neurons and in a linear pattern immediately adjacent to NFH+ axons. CLIC4 was detected sparsely in nuclei of GLUL+ and GFAP+ astrocytes and strongly in discrete cytosolic regions of IBA1+ microglia. In contrast to published findings with D2 mice, acute ethanol (4g/kg IP) did not alter *Clic4* mRNA expression in PFC of male C57BL/6J (B6) mice; however, expression levels were increased by 137% in females ($p < 0.01$). Pan-neuronal deletion of *Clic4* in PFC decreased daily voluntary ethanol consumption in a 5-week 3-bottle-choice (15% v/v, 30% v/v, or water) intermittent access study by 15% in female mice ($p = 0.01$) and 12% in males ($p = 0.03$). While ethanol concentration preference was unaffected by deletion in males ($p = 0.37$), females showed a 20% decrease in preference for the lower concentration ethanol ($p < 0.01$). In contrast, oligodendrocyte-specific deletion of *Clic4* led to a 14% increase in average daily ethanol intake in female mice ($p < 0.01$) but did not affect intake in males ($p = 0.15$). This was accompanied by a 28% increase in preference for the lower concentration ethanol in females ($p <$

0.01) and an 11% increase in males (p = 0.02). Considering its abundant expression in oligodendrocytes, inducibility by acute ethanol, and capability of modulating ethanol consumption differentially in multiple cell types, *Clic4* may play an important role in the brain's molecular response to ethanol and the development of dysfunctional ethanol-related behaviors.

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Poster

237. Genetic and Molecular Mechanisms Underlying Alcohol Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 237.09/U11

Topic: G.08. Drugs of Abuse and Addiction

Title: Cortical proteomic profiles associated with ethanol-induced impulsivity and reward seeking behaviors in mice

Authors: *P. A. STARSKI[1], S.-I. HONG[4], L. PEYTON[2], A. OLIVEROS[3], K. WININGER[6], S. KANG[5], D.-S. CHOI[4];
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Abstract: Impulsivity is a risk factor for many psychiatric disorders including alcohol use disorder (AUD). Highly impulsive individuals are more vulnerable to alcohol abuse due to increased risk taking behaviors. However, it is not well understood whether binge alcohol use increases the propensity for impulsive behavior and its effect on the anterior cingulate cortex (ACC), a main brain region encoding impulsivity. In this study, we utilized a novel experimental paradigm demonstrating that binge-like ethanol exposure progressively leads to maladaptive impulsive behavior. For testing waiting impulsivity, we employed the 5-choice serial reaction time task (5-CSRTT) in C57BL/6J male mice, a task strongly correlated with the anterior cingulate cortex. We assessed premature responses during reward seeking in fixed and variable inter-trial interval (ITI) 5-CSRTT sessions. We further characterized our ethanol-induced impulsive mice using the open field, y-maze, 2-bottle choice, and an action-outcome task. Finally, we performed label-free proteomic analysis of the ACC to observe protein changes in ethanol-induced impulsive mice compared to untreated mice. Our results demonstrated that binge-like ethanol exposure significantly increased premature responses during variable ITI sessions during a prolonged abstinent period. Ethanol treated mice exhibited anxiety-like behavior during their impulsivity testing and was observed again after twenty days of treatment abstinence. Ethanol treated mice were displaying signs of anhedonia through decreased

motivation for a sucrose reward compared to air-exposed control mice, while also demonstrating reduced responses during devaluation testing. Finally, ingenuity pathway analysis revealed a significant change of several impulsivity-related proteins such as KCNIP3 (potassium voltage-gated channel interacting protein 3) and CACNG2 (calcium voltage-gated channel subunit gamma 2). Furthermore, ingenuity pathway analysis showed that mTOR (mechanistic target of rapamycin) canonical pathway to be highly affected in ethanol-induced impulsive mice compared to control mice. Overall, our findings indicate that ethanol treated mice increased impulsive behavior, but reduced hedonic behavior for the sucrose reward. Proteomic profiles of ethanol-induced impulsive mice may explain molecular mechanisms underpinning maladaptive impulsive behavior

Disclosures: P.A. Starski: None. **S. Hong:** None. **L. Peyton:** None. **A. Oliveros:** None. **K. Wininger:** None. **S. Kang:** None. **D. Choi:** None.

Poster

237. Genetic and Molecular Mechanisms Underlying Alcohol Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 237.10/U12

Topic: G.08. Drugs of Abuse and Addiction

Support: NIAAA R37AA01684 (DR)

Title: The BDNF valine 68 to methionine polymorphism decreases alcohol reward sensitivity, causes alcohol-dependent recognition memory deficits and reduces alcohol's anxiolytic effects in mice

Authors: *Y. EHINGER[1], J. J. MOFFAT[2], S. A. SAKHAI[3], D. RON[4];
[1]Univ. of California San Francisco, San Francisco, CA; [2]Dept. of Neurol., [3]Neurol., Univ. of California, San Francisco, San Francisco, CA; [4]Dept. of Neurol., UCSF, San Francisco, CA

Abstract: Brain-derived neurotrophic factor (BDNF) is a neuronal modulator that plays a key role in neuronal survival, development and plasticity [1]. A common human polymorphism in this gene, Val66Met, which decreases the activity-dependent release of BDNF [2], has been associated with a number of psychiatric disorders [1]. As alcohol addiction is one of the most prevalent psychiatric diseases, we set to determine whether the BDNF polymorphism is associated with alcohol use disorder (AUD). To do so, we created a transgenic mouse line expressing the mouse homolog of the human polymorphism (Val68Met) and found that the Met68BDNF mice consume alcohol despite negative consequences, a phenotype that was reversed by systemic administration of the TrkB agonist, LMA22A-4 [3]. Here, we set out to determine whether Met68BDNF mice exhibit other alcohol-dependent behavioral deficits as compared to wild-type (WT) Val68BDNF mice. Using a conditioned place preference paradigm,

we found that the Met68BDNF polymorphism reduces alcohol place preference, suggesting that the Met68BDNF mice are less sensitive to the rewarding properties of alcohol. Using the novel object recognition test, we also observed that a moderate dose of alcohol (1.25 g/kg) results in impaired recognition memory in Met68BDNF mice, but not in Val68BDNF animals. Finally, we tested whether the Met68BDNF polymorphism alters the anxiolytic effects of alcohol using the elevated plus-maze task. When given a moderate, anxiolytic dose (1.25 g/kg) of alcohol, Met68BDNF mice spend less time in the distal arms of the maze, compared to Val68BDNF carriers, suggesting reduced sensitivity to the anxiolytic effects of alcohol. This phenotype was reversed by overexpressing the WT Val68BDNF gene in the ventral hippocampus of Met68BDNF mice. As the ventral hippocampus has been associated with stress, memory and reward (4), it is plausible that impairment in BDNF signaling in this brain region mediates the alcohol-dependent deficits described above. References 1. Song et al., Mol. Psych., 2017 2. Egan et al., Cell, 2003 3. Warnault et al., Bio. Psych., 2016 4. Fanselow and Dong, Neuron, 2010 Supported by NIAAA R37AA01684 (DR)

Disclosures: **Y. Ehinger:** None. **J.J. Moffat:** None. **S.A. Sakhai:** None. **D. Ron:** None.

Poster

237. Genetic and Molecular Mechanisms Underlying Alcohol Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 237.11/U13

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH K99 AA025677

Title: Circuit-specific CRISPR/Cas9 gene editing reveals an extended amygdala neuropeptide receptor signaling mechanism driving alcohol drinking, anxiety, and avoidance

Authors: *W. J. GIARDINO, H. YAMAGUCHI, L. DE LECEA;
Psychiatry & Behavioral Sci., Stanford Univ., Stanford, CA

Abstract: Lateral hypothalamus (LH) neurons containing the neuropeptide hypocretin (Hcrt; orexin) profoundly influence motivated behavior, and Hcrt interactions with amygdala and mesolimbic neural pathways drive hyperarousal associated with substance abuse (including insomnia, anxiety, and compulsive reward-seeking). We investigated Hcrt-LH neurocircuits in alcohol addiction by measuring and perturbing Hcrt interactions with specific limbic neuronal populations in a mouse model of free-choice chronic binge drinking that captures key aspects of human alcohol use disorder. First, we identified transcriptional activation of Hcrt-LH neurons associated with alcohol withdrawal-induced anxiety behavior, and used *in vivo* Ca^{2+} recordings to reveal heightened sensitivity of *Hcrt*-LH neurons to aversive stimuli during withdrawal. To delineate candidate circuit mechanisms, we interrogated Hcrt-LH neurons projecting to either the

bed nuclei of stria terminalis (BNST) or ventral tegmental area (VTA), regions expressing G-protein-coupled Hcrt receptors (HcrtR1, HcrtR2). To investigate Hcrt-BNST functional interactions, we combined pharmacological or transgenic manipulations of the Hcrt system with *in vivo* optogenetic stimulation of distinct BNST subpopulations (corticotropin-releasing factor, *Crf*; or cholecystokinin, *Cck*). These studies uncovered the necessity of HcrtR signaling and Hcrt-LH neurons for behavioral avoidance driven by *Crf*-BNST neurons, but not behavioral preference driven by *Cck*-BNST neurons, suggesting that negative affective consequences of chronic alcohol exposure may rely on *Crf*-BNST-HcrtR signaling. To directly test cell type-specific HcrtR signaling in chronic alcohol drinking, we developed a CRISPR-Cas9 gene editing system to induce fixed mutations in HcrtR genes within Cre-defined BNST and VTA subpopulations of adult mice. We found that genetic disruption of HcrtR1 specifically in *Crf*-BNST neurons strongly reduced alcohol intake, as well as anxiety and avoidance behaviors. In conclusion, we propose a specific and essential role for *Crf*-BNST-HcrtR1 signaling in maintaining excessive alcohol drinking through negative affective processes linked to dysregulated hyperarousal.

Disclosures: W.J. Giardino: None. **H. Yamaguchi:** None. **L. de Lecea:** None.

Poster

237. Genetic and Molecular Mechanisms Underlying Alcohol Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 237.12/U14

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH-NIAAA R01 AA027 (DR)

Title: mTORC1-dependent translation of microRNA machinery genes in the nucleus accumbens of mice excessively consuming alcohol

Authors: *E. L. WHITELEY, S. LAGUESSE, F. LIU, D. RON;
Dept. of Neurol., Univ. of California, San Francisco, San Francisco, CA

Abstract: The mechanistic target of rapamycin complex 1 (mTORC1) plays a crucial role in dendritic protein translation which influences synaptic plasticity, learning and memory [1]. We previously reported that increased activation of the H-Ras/PI3K/Akt signaling pathway following excessive alcohol consumption results in the activation of mTORC1 in the nucleus accumbens (NAc) of rodents [2-7]. We further found that mTORC1 activation in the NAc drives morphological and synaptic adaptations in response to alcohol through the translation of synaptic proteins [8, 9]. Importantly, inhibition of mTORC1 signaling with rapamycin reduced alcohol consumption, preference and relapse [2, 5, 7, 10]. Together, these findings indicate that mTORC1 is a pivotal factor in mechanisms underlying the development and/or maintenance of

alcohol drinking behaviors. In order to identify alcohol-mediated, mTORC1-dependent gene translation, we employed RNA Seq analysis. Mice underwent an intermittent access to 20% alcohol using a two bottle choice paradigm for 7 weeks, with control animals drinking water only, before being treated with rapamycin or vehicle 3 hours prior to euthanization. Polysomes, consisting of actively translating RNA bound to ribosomes, were isolated from the NAc of mice prior to RNA Seq. Three of the 12 transcripts whose translation was increased by alcohol in an mTORC1-dependent manner were involved in gene silencing processes; Cnot4, Tsnax and Trnc6a. RNAseq data were confirmed by RT-PCR for Cnot4 and Tsnax and but not for Trnc6a. We and others previously found that microRNAs (miRs), small non-coding RNAs that promote mRNA degradation and/or repression of translation of target genes , are elevated by alcohol in the mPFC of rodents to promote excessive drinking and dependence [12, 13]. We therefore hypothesized that alcohol, by upregulating the mTORC1/miR axis in the NAc, downregulates the expression and/or translation of genes. To elucidate potential miRs target genes, we analyzed the polysomal RNA Seq data for genes that decreased by alcohol relative to water-drinking controls in a mTORC1-dependent manner. Our analysis identified 16 mRNAs that answered these criteria. Utilizing computational sequence-based miR prediction software, TargetScan, we found that several of these candidate mRNAs are putative targets for miRs previously reported to be elevated in the brain following alcohol dependence. Together, our data suggests that activation of mTORC1 in the NAc following alcohol consumption may contribute to miR-mediated gene silencing.

Disclosures: **E.L. Whiteley:** None. **S. Laguesse:** None. **F. Liu:** None. **D. Ron:** None.

Poster

237. Genetic and Molecular Mechanisms Underlying Alcohol Dependence

Location: Hall A

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Program #/Poster #: 237.13/U15

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH/NIAAA R01 AA025038
NSF NRT: Neurophotonics 1633516

Title: Involvement of prelimbic cortex pituitary adenylate cyclase-activating polypeptide in excessive drinking

Authors: *M. A. MINNIG[1], S. G. QUADIR[1], A. EVANS-STRONG[2], M. RILEY[2], P. COTTONE[1], V. SABINO[1];
[1]Boston Univ. Sch. of Med., Boston, MA; [2]Northeastern Univ., Boston, MA

Abstract: Projections from the prefrontal cortex to the striatum have been proposed to mediate the pathological drive to drink compulsively during alcohol dependence despite adverse

consequences. Here, we investigate the role of the neuropepeptide pituitary adenylate cyclase-activating polypeptide (PACAP) and its receptor PAC1R on compulsive alcohol intake. Based on preliminary data from our lab and others, we hypothesize that overactivation of this system in the prelimbic prefrontal cortex (PrL) to nucleus accumbens core (NAcc) pathway mediates excessive drinking. We first characterized the PACAP population as glutamatergic long-range projecting cells in layer 2/3 of PrL using fluorescent *in situ* hybridization. We also confirmed the projection of these cells from PrL to NAcc using injection of a cre-dependent reporter virus into the PrL in PACAP-cre mice, which showed visible fibers in NAcc and other areas relevant to alcohol drinking. Subsequently, in order to model chronic alcohol exposure, we used an Intermittent Access to Two Bottle Choice model of alcohol drinking. In mice exposed to eight weeks of alcohol versus control animals, we found an increase in the amount of activation in PrL and NAcc Core using FosB immunohistochemistry, and found no significant difference in activation in the neighboring infralimbic cortex or nucleus accumbens shell. We followed this with a co-localization study for Fos protein with PACAP and PAC1R mRNA in the PrL and NAcc, respectively. To extend this data, we used a dual-virus designer-receptor approach to activate or inhibit the PACAPergic cells of the PrL to study the effect on excessive and compulsive alcohol consumption Further, we used PACAP-cre mice and a cre-dependent caspase virus to selectively ablate only PACAP expressing cells in the prelimbic cortex prior to alcohol exposure. Last, we injected a short hairpin RNA virus targeted to the PAC1R receptor into the NAcc prior to alcohol exposure, to assess whether PAC1R knockdown blocks excessive and compulsive drinking. Overall, we show a projection from PACAP expressing cells in prelimbic cortex to the nucleus accumbens core, and the effect of this projection on aberrant alcohol drinking in a mouse model of chronic alcohol exposure.

Disclosures: **M.A. Minnig:** None. **S.G. Quadir:** None. **A. Evans-Strong:** None. **M. Riley:** None. **P. Cottone:** None. **V. Sabino:** None.

Poster

237. Genetic and Molecular Mechanisms Underlying Alcohol Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 237.14/U16

Topic: G.08. Drugs of Abuse and Addiction

Support: AA025244
AA027293

Title: Differential regulation of excessive alcohol consumption by the transcriptional regulator LMO4

Authors: ***R. MAIYA**[1], M. B. POMRENZE[3], T. TRAN[4], G. R. TIWARI[4], R. D. MAYFIELD[2], R. O. MESSING[4];

[1]Neurosci., [2]Waggoner Ctr. for Alcohol and Addiction Res., The Univ. of Texas At Austin, Austin, TX; [3]Neurosci., Univ. of Texas at Austin, Austin, TX; [4]The Univ. of Texas at Austin, Austin, TX

Abstract: Repeated alcohol exposure leads to changes in gene expression that are thought to underlie the transition from moderate to excessive drinking. However, the mechanisms by which these gene expression changes are mobilized to a maladaptive response are not well understood. One mechanism could involve the recruitment of transcriptional co-regulators that bind and modulate the activity of several transcription factors. Our results indicate that the transcriptional regulator *Lim-Only 4* (LMO4) is one such candidate regulator. Mice harboring a gene trap insertion at the *Lmo4* locus (*Lmo4gt/+)* consumed significantly more and showed enhanced preference for alcohol in a 24-hour intermittent access procedure. shRNA-mediated knockdown of LMO4 in the nucleus accumbens (NAc) enhanced alcohol consumption whereas knockdown in the basolateral amygdala (BLA) led to decreased alcohol consumption and preference as well as reduced conditioned place preference to alcohol. To identify transcriptional targets of LMO4 in the BLA and NAc, we carried out unbiased transcriptome profiling of these two brain regions in WT and *Lmo4gt/+*mice. Of the 1000 differentially expressed genes identified in each brain region, only 48 were common suggesting that transcription targets of LMO4 are vastly different between the two brain regions. We next took a systems approach to identify gene networks that were responsive to LMO4 downregulation in the BLA. Weighted gene co-expression network analysis revealed genes related to the extracellular matrix and the kappa opioid receptor (*Oprk1*) are important transcriptional targets of LMO4 in the BLA. Consistent with these findings, disruption of the ECM in the BLA using the enzyme chondroitinase ABC significantly reduced alcohol consumption. Chromatin immunoprecipitation studies indicated that LMO4 bound to the kappa opioid receptor (KOR) promoter in the amygdala and that infusion of Nor-BNI, a selective antagonist of KOR into the BLA reduced alcohol consumption. Future experiments will determine how LMO4 itself and the LMO4-regulated transcriptome change with alcohol consumption.

Disclosures: R. Maiya: None. **M.B. Pomrenze:** None. **T. Tran:** None. **G.R. Tiwari:** None. **R.D. Mayfield:** None. **R.O. Messing:** None.

Poster

237. Genetic and Molecular Mechanisms Underlying Alcohol Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 237.15/U17

Topic: G.08. Drugs of Abuse and Addiction

Support: NIAA R21AA015598

Title: Characterization of the G protein coupled receptor kinase 2 in alcohol induced behaviors in *Drosophila melanogaster*

Authors: *Y. KANG[1], E. ENGDORF[2], T. T. MCKENZIE[3], R. DEL ABRA[2], G. W. ROMAN[4]; [1]Natural Sci., [2]Univ. of Houston Downtown, Houston, TX; [3]Univ. of Houston-Downtown, Houston, TX; [4]Univ. of Mississippi, Oxford, MS

Abstract: G protein Couple Receptor Kinase (GRK) superfamily are conserved between invertebrates and vertebrates. While the GRK2/3 subfamily is relatively well characterized in its function in drug tolerance and addiction, the involvement of GRK4/5/6 subfamily in drug tolerance and addiction remains elusive. Here we report the role of *Drosophila* G protein Coupled Receptor Kinase 2 (GPRK2) in mediating alcohol sensitivity and its role in locomotion modulation and arousal state. Specifically, flies with no neuronal expression of GPRK2 is more sensitive to the sedating effect of alcohol. In addition, *gprk2* mutant flies do not develop alcohol rapid tolerance, a phenotype consistent with the role of GRK in regulating receptor desensitization and internalization. Lastly, we have mapped the requirement of *gprk2* to the central complex in mediating locomotive behaviors, possibly through the Dopamine signaling pathway.

Disclosures: Y. Kang: None. **E. Engdorf:** None. **T.T. McKenzie:** None. **R. Del Abra:** None. **G.W. Roman:** None.

Poster

237. Genetic and Molecular Mechanisms Underlying Alcohol Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 237.16/U18

Topic: G.08. Drugs of Abuse and Addiction

Support: NIAAA AA022448 S1
AFPE Pre-doctoral fellowship
USC School of Pharmacy

Title: P2X4 receptor cross-talk in the VTA: Implications in alcohol addiction and drug development

Authors: *L. RODRIGUEZ[1], C. YOU[3], J. J. WOODWARD[4], M. S. BRODIE[5], D. L. DAVIES[2];
[1]Pharmacol. and Pharmaceut. Sci., [2]Titus Family Dept of Clin. Pharm., USC, Los Angeles, CA; [3]Univ. of Illinois At Chicago, Chicago, IL; [4]Neurosciences, Med. Univ. of South Carolina, Charleston, SC; [5]Dept Physiol & Biophys M/C901, Univ. Illinois-Chicago, Chicago, IL

Abstract: Purinergic receptors (P2XRs) are an emerging target for the development of drugs to prevent/treat several CNS disorders, including alcohol use disorder (AUD). For example, genetic, pharmacological and behavioral mouse studies report an inverse relationship between ethanol (EtOH) intake and P2X4R activity, although the underlying mechanism for this phenomenon is not understood. Several P2X receptors are capable of receptor *cross-talk*: an interaction where one receptor (e.g. *P2X2*) influences the activity of another (e.g. *GABA* or *AMPA* receptors.) However, the physiological and/or behavioral relevance of P2XR cross-talk remains unknown. In preliminary studies, we tested for interactions between P2X4Rs and ionotropic glutamate (*NMDA*) receptors, which are a central component of addiction circuitry and regulator of dopamine cell firing. Two-electrode voltage-clamp electrophysiology experiments in *X. laevis* oocytes demonstrated that P2X4 and NMDA receptors co-express and function individually, yet simultaneous, *co-activation* of P2X4Rs and NMDARs produced significantly lower responses than the sum of the individual receptor responses (*cross-talk.)* Both P2X4 and NMDA receptors are expressed in the Ventral Tegmental Area (VTA) of the brain, which plays a role in ethanol-induced signaling and addiction. Therefore, we hypothesize that, in the VTA, P2X4R cross-talk regulates NMDA receptor activity, and that EtOH dysregulates cross-talk, which contributes to the development of AUD. In this study, we first performed brain slice electrophysiology experiments to characterize the effects of EtOH on P2X4Rs in the VTA region of the brain. Next, we determined the role of P2X4-NMDA receptor cross-talk in dopaminergic and GABAergic neurons using an *ex vivo* receptor knockdown method (**Si**-RNA **L**oaded **E**lectrodes k**N**ockdown **T**arget, or *Si-LENT* slice.) To better understand how EtOH dysregulates cross-talk, we incorporated mutant EtOH-resistant NMDA and/or P2X4 receptors in TEVC cross-talk studies. To explore the mechanism for P2X4-NMDAR cross-talk, we transfected fluorescently labeled receptors into mammalian cells and performed confocal microscopy experiments. Overall, our findings indicate that P2X4 receptors play a previously unrecognized role in neuronal cell firing within the VTA, and provide evidence for the behavioral function of P2X4 receptors, in the context of alcohol consumption. The findings suggest a novel mechanism for the regulation of NMDARs in the VTA region of the brain and provides a potential target (P2X receptor cross-talk) for the treatment of neurological diseases, including AUD.

Disclosures: L. Rodriguez: None. **C. You:** None. **J.J. Woodward:** None. **M.S. Brodie:** None. **D.L. Davies:** None.

Poster

237. Genetic and Molecular Mechanisms Underlying Alcohol Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 237.17/U19

Topic: G.08. Drugs of Abuse and Addiction

Support: MEXT KAKENHI, Grant Number 16K08913

Title: Comprehensive analysis of miRNA expression in ethanol dependence in mice

Authors: *K. MIZUO, T. YAMAGUCHI, S. WATANABE;
Sapporo Med. Univ., Sapporo, Japan

Abstract: Alcoholism is a complex disorder resulting from multiple interaction between genetic, epigenetic and environmental factors. Alcohol dependence, a major symptom in the alcoholism, has spread worldwide, and the number of the patient is over a million in Japan. Although a lot of study suggested several candidates, certain mechanism underlying the development of ethanol dependence is still unclear. Several microRNAs (miRs) are highly expressed in the central nervous system and modulate the brain function via regulating gene expression. We have reported that acute ethanol administration caused the long-lasting increase in the expression of miR-124 in mouse brain. In the present study, we investigated the expression of miRs in mouse ethanol dependence model. Mice were treated with liquid diet containing ethanol for 10 days. Using the escalating ethanol dosage schedule, the mice were fed the ethanol diet as follows: 1st day: 1 w/v%; 2nd and 3rd day: 3 w/v%; 4th to 10th day: 4 w/v% ethanol diet, respectively. The control mice were given the same volume of ethanol-free liquid diet with sucrose substituted in isocaloric quantities for ethanol. The mice chronically treated with ethanol revealed severe withdrawal signs after discontinuation of ethanol. Total RNA was extracted from mouse limbic forebrain (containing nucleus accumbens). Comprehensive analysis of miRNA expression in ethanol dependence was performed by miRNA array. The miRNA array analysis showed 47 upregulated miRNAs in the ethanol dependence. Especially, miR-3063-3p highly upregulated (fold change >50) in ethanol dependence. Using target predictions of miRNA, we found histone deacetylase 7 as a target of miR-3063-3p. We found that the treatment of a class II histone deacetylase inhibitor trichostatin A during ethanol withdrawal significantly suppressed the ethanol induced rewarding effect. Our findings suggest that the upregulation of miR-3063-3p, resulting in the changes in histone deacetylase, has an important role in the development of alcohol dependence.

Disclosures: K. Mizuo: None. **T. Yamaguchi:** None. **S. Watanabe:** None.

Poster

237. Genetic and Molecular Mechanisms Underlying Alcohol Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 237.18/U20

Topic: G.08. Drugs of Abuse and Addiction

Title: Estrogen receptor alpha and beta modulation of ethanol withdrawal-induced anxiety

Authors: *L. ENGEL, E. HOWELL, P. J. CURRIE;
Reed Col., Portland, OR

Abstract: Previous research suggests that female rats drink more than males in an estrous-dependent manner, however, they also exhibit fewer anxiety-like behaviors in response to ethanol withdrawal, compared to males. These differences have been shown to be regulated by estradiol 17β (E2) however, the mechanism mediating the effects of E2 are currently unknown. The present study investigated the role of estrogen receptors alpha (ERα) and beta (ERβ) in modulating ethanol withdrawal-induced anxiety in female rats. Animals were divided into the following groups: sham-operated, ovariectomized, ovariectomized and treated with the ERα agonist PPT, ovariectomized and treated with the ERβ agonist DPN, and ovariectomized and treated with PPT and DPN (n=6 for each group). Rats were then habituated to ethanol for 6 weeks via a 2-bottle intermittent access paradigm. PPT (1 mg/kg), DPN (1 mg/kg), and sesame oil vehicle were administered for 5 days, starting the day before the last ethanol exposure. On the final day of drug administration, 72 hours post-ethanol exposure, rats underwent behavioral testing on the elevated plus maze (EPM) and open field (OF) test. All OVX rats drank less than the sham-operated controls when controlled for body weight and exhibited a greater anxiety-like response to ethanol withdrawal on both the EPM and OF tests. Treatment with PPT and DPN attenuated this increased anxiety-like response in the EPM but not OF test. Administration of both agonists had an additive effect, reducing the anxiety-like response further than either agonist administered alone, suggesting that activation of both ERα and ERβ is important for E2's effects on ethanol withdrawal-induced anxiety, and that this activation may be a driving factor behind observed sex differences during withdrawal.

Disclosures: L. Engel: None. **E. Howell:** None. **P.J. Currie:** None.

Poster

237. Genetic and Molecular Mechanisms Underlying Alcohol Dependence

Location: Hall A

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Program #/Poster #: 237.19/U21

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R01DA035958
NIH Grant F32AT009945

Title: Peripheral mechanoreceptor activation modulates mesolimbic GABA and dopamine neurons and ameliorates withdrawal symptoms in ethanol dependent rats

Authors: *K. BILLS, K. CREECH, J. PRUETT, D. OBRAY, S. C. STEFFENSEN;
Brigham Young Univ., Provo, UT

Abstract: The therapeutic benefits attributed to activation of peripheral mechanoreceptors are poorly understood. There is growing evidence that mechanical stimulation (MStim) modulates substrates in the supraspinal central nervous system (CNS) that are outside the canonical somatosensory circuits. The aim of this study was to evaluate the effects of MStim applied to the cervical spine on neurons and neurotransmitter release in the mesolimbic dopamine (DA) system, an area implicated in reward and motivation. Utilizing electrophysiological, pharmacological and neurochemical techniques, in male Wistar rats, we demonstrate that low frequency (45-80 Hz), but not higher frequency (115 Hz), peripheral MStim to the lower cervical spine depresses firing of ventral tegmental area (VTA) GABA neurons (52.8% baseline; 450 sec) and increases VTA DA firing (248% baseline; 500 sec) and basal (178.43 % peak increase at 60 min) and evoked DA release in the nucleus accumbens (NAc; 135.03 % peak increase at 40 min). Furthermore, MStim-induced DA release was mediated, in part, by endogenous opioid and acetylcholine release in the NAc. Additionally, peripheral mechanosensory stimulation provides protection against chronic ethanol withdrawal symptoms and dependence-induced insensitivity of VTA GABA neurons to ethanol reintroduction. These findings demonstrate robust effects of MStim on CNS substrates not typically associated with somatosensory circuits and suggest the need to explore more broadly the extra-somatosensory effects of peripheral mechanoreceptor activation and the specific role for mechanoreceptor-based therapies in the treatment of substance abuse.

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Poster

237. Genetic and Molecular Mechanisms Underlying Alcohol Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 237.20/U22

Topic: G.08. Drugs of Abuse and Addiction

Support: NIHAA021657, AA022292

Title: Deficient endocannabinoid signaling in the lateral habenula underlies hyperalgesia during alcohol withdrawal

Authors: *J. YE[1], R. FU[2], Y. TANG[1], X. CHEN[2], Y. ZOU[2], W. ZUO[3], A. BEKKER[2]; [2]Anesthesiol., [1]Rutgers, The State Univ. of New Jersey, New Jersey Med. Sch., Newark, NJ; [3]Anesthesiol., Rutgers, The State Univ. of New Jersey, New Jersey Med. Sch., Newark, NJ

Abstract: Repeated excessive alcohol exposure and withdrawal are associated with hyperalgesia. However, the molecular and cellular mechanisms underlying this association are unclear. The lateral habenula (LHb) has been implicated in the coding of the pathophysiology of

drugs of abuse and pain. The endocannabinoid signaling plays a significant role in the regulation of neuronal excitability in many brain areas including the LHb. Here, we investigated the role of endocannabinoid signaling in the LHb on hyperalgesia during alcohol withdrawal and on alcohol intake using animal models of ethanol dependence. The nociceptive sensitivity was increased in adult male Long-Evans rats at 24 h withdrawal from chronic alcohol administration, which was alleviated by intra-LHb injection of URB597 (fatty acid amide hydrolase inhibitor) or JZL184 (MAGL inhibitor). MAGL is a key clearance enzyme of endocannabinoids. Intra-LHb URB597 or JZL184 also reduced alcohol intake both in the homecage and the operant chamber upon reaccess. Also, in the LHb of alcohol-withdrawal rats, mRNA level of *Magl* and *Daglb*, and protein expression of MAGL was increased, but the protein expressions of diacylglycerol lipase alpha (DAGL-a) and CB1 receptors were decreased. The present results suggest a key role for endocannabinoid signaling in the neuroadaptations during chronic alcohol exposure and withdrawal, in which a deficiency in LHb endocannabinoid signaling contributes to hyperalgesia and excessive alcohol consumption.

Disclosures: **J. Ye:** None. **R. Fu:** None. **Y. Tang:** None. **X. Chen:** None. **Y. Zou:** None. **W. Zuo:** None. **A. Bekker:** None.

Poster

237. Genetic and Molecular Mechanisms Underlying Alcohol Dependence

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Program #/Poster #: 237.21/U23

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant AA027171
NIH Grant AA027660

Title: Upregulation of the sodium calcium exchanger precedes the onset of alcohol withdrawal seizures

Authors: ***P. N'GOUEMO**[1], L. R. AKINFIRESOYE[1], J. NEWTON[1], S. SUMAN[2], K. DATTA[2];
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Abstract: The inferior colliculus (IC) is critical in the initiation of acoustically evoked alcohol withdrawal seizures (AWSs), and altered Ca^{2+} signaling is thought to contribute to the pathogenesis and pathophysiology of AWSs. We have previously reported that Ca^{2+} influx through $Ca_V1.3$ L-type Ca^{2+} channel plays a role in the occurrence—but not necessarily in the initiation—of AWSs. Increased intracellular Ca^{2+} ($[Ca^{2+}]_i$) plays an important role in the pathogenesis of seizures; $[Ca^{2+}]_i$ is regulated by intracellular Ca^{2+}-buffering proteins and Ca^{2+} extrusion via transport/exchanger at the cell surface. One such transporter/exchanger is the

Na^+/Ca^{2+} exchanger (NCX) that operates in the forward mode or reverse mode to drive either Ca^{2+} extrusion/Na^+ entry or Ca^{2+} entry/Na^+ extrusion, respectively. Here, we examine the putative role of Ca^{2+} influx via NCX in the pathogenesis and pathophysiology of AWSs by quantifying mRNA and protein expression associated with NCX type 1 (NCX1), 2 (NCX2) and 3 (NCX3) in male Sprague-Dawley rat IC neurons at various time points during the alcohol withdrawal period. Quantitative RT-PCR analysis shows that NCX1 but not NCX2 and NCX3 mRNA expression was markedly elevated 3 h during the alcohol withdrawal period when no seizure susceptibility is observed, compared to controls. Expression of NCX1 mRNA remains elevated at 24 h during the alcohol withdrawal when the seizure susceptibility is maximal and returns to control levels at 48 h when the seizure susceptibility is resolved, as compared to controls. Expression of NCX2 and NCX3 mRNA was increased 24 h following alcohol withdrawal and returned to control levels at 48 h. Western blot analysis reveals that NCX1 protein expression was significantly increased 3 h and 24 h following alcohol withdrawal compared to controls. In contrast, NCX3 protein expression was increased 3 h following alcohol withdrawal compared to controls, but return to control levels by the 24^{th} h following alcohol withdrawal. No significant change was observed in NCX2 protein expression during alcohol withdrawal. Thus, upregulated NCX1 mRNA expression 3 h into the alcohol withdrawal was accompanied by a corresponding increase in protein expression, thus preceding the onset of AWS susceptibility. The elevated NCX1 subunit mRNA and protein expression 24 h following alcohol withdrawal parallels the occurrence of AWSs. These findings suggest that upregulation of NCX1 expression likely contributes to the pathogenesis and pathophysiology of AWSs.

Disclosures: P. N'Gouemo: None. **L.R. Akinfiresoye:** None. **J. Newton:** None. **S. Suman:** None. **K. Datta:** None.

Poster

237. Genetic and Molecular Mechanisms Underlying Alcohol Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 237.22/U24

Topic: G.08. Drugs of Abuse and Addiction

Support: NIAAA P50 AA022538
U01 AA020912

Title: The HDAC inhibitor SAHA alleviates depression-like behavior and normalizes HDAC2 and acetylated histone H3 levels in the hippocampus during alcohol withdrawal

Authors: *W.-Y. CHEN[1], H. ZHANG[2], E. GOTTA[1], H. CHEN[4], S. C. PANDEY[1], A. W. LASEK[3];
[2]Dept Psychiatry, [3]Psychiatry, [1]Univ. of Illinois at Chicago, Chicago, IL; [4]Psychiatry, UIC, Chicago, IL

Abstract: Withdrawal from chronic alcohol drinking leads to a negative affective state defined by depression, anxiety and anhedonia. This negative affective state can contribute to relapse to alcohol abuse, thus continuing the cycle of addiction. Alcohol withdrawal causes changes in acetylation of the N-terminal tails of histone proteins in the brain, leading to alterations in the expression of key genes involved in depression. These epigenetic neuroadaptations are also observed in stress-induced models of depression. Notably, treatment with histone deacetylase (HDAC) inhibitors reduces depression-like behavior in rodents. The purpose of this study was to examine the levels of acetylation of histone H3 lysine 9 (H3K9ac) and expression of 10 HDAC genes in the hippocampus, an alcohol-sensitive region of the brain involved in depression, and to determine if treatment with a pan-histone deacetylase inhibitor, suberoylanilide hydroxamic acid (SAHA) can alter H3K9ac and alleviate depression-like behavior during withdrawal from chronic alcohol drinking. Male Sprague-Dawley rats were treated with the Lieber-DeCarli ethanol liquid diet for 15 days and then underwent alcohol withdrawal for 24 hours. Total H3K9ac levels were decreased in the hippocampal CA3 region during withdrawal as measured by immunogold labeling. Corresponding increases in HDAC2 (mRNA and protein) and HDAC6 (mRNA) also occurred during withdrawal. Treatment with SAHA during withdrawal normalized levels of both H3K9ac and HDAC2 protein. We next examined depression-like behavior during withdrawal using the sucrose splash and sucrose preference tests. Withdrawal from chronic alcohol drinking decreased grooming time and sucrose preference, indicative of depression. Treatment with SAHA during withdrawal alleviated depression-like behavior. These results demonstrate that withdrawal from chronic alcohol drinking causes epigenetic alterations in the hippocampus and depression-like behavior that can be normalized by treatment with an HDAC inhibitor. This investigation provides a novel epigenetic-based target for the treatment of alcohol withdrawal-related depression.
This study is supported by NIAAA P50 AA022538 (to AWL and SCP) and U01 AA020912 (to AWL).

Disclosures: W. Chen: None. **H. Zhang:** None. **E. Gotta:** None. **H. Chen:** None. **S.C. Pandey:** None. **A.W. Lasek:** None.

Poster

237. Genetic and Molecular Mechanisms Underlying Alcohol Dependence

Location: Hall A

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Program #/Poster #: 237.23/U25

Topic: F.01. Neuroethology

Support: NIH/NIMHD 2G12MD007592
NIH/NIAAA R15AA020996
NIH/NIGMS 2R25GM069621-14.

Title: Social environments alters alcohol responses

Authors: *C. M. SIERRA, V. VALLES, P. SABANDAL, K.-A. HAN;
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Abstract: Animal behaviors such as mating, eating and reward-seeking are continually influenced by social environment. Its underlying neurobiological basis, however, remains unclear. Here, we investigated the effect of different social environments on various ethanol responses in *Drosophila*. The *Drosophila* model allows to study ethanol-associated behaviors including sedation, tolerance, euphoria, behavioral disinhibition and sensitization. To identify the mechanism by which social environment affects ethanol responses, we compared the socially isolated versus socially enriched conditions in the wild-type Canton-S flies. We found that socially isolated flies were less sensitive to the sedative and euphoric effects of ethanol compared to socially enriched flies. To pinpoint whether the effect of social environment on sedation and euphoria is due to the social experience during housing or at the time of ethanol exposure, we compared the following conditions: (1) singly housed/singly exposed versus group housed/singly exposed and (2) singly housed/group exposed versus group housed/group exposed flies. We found that group exposed flies consistently displayed greater ethanol-induced euphoric response compared to singly exposed flies regardless of housing conditions. This suggests that the social environment during ethanol exposure, but not housing, is the major factor influencing the euphoric response. We will present the progress of this study and the role of dopamine D2 receptor as a key factor mediating social environment information. Findings of this study may have novel implications for social environment-dependent alcohol use or addiction.

Disclosures: C.M. Sierra: None. **V. Valles:** None. **P. Sabandal:** None. **K. Han:** None.

Poster

238. Addiction Treatment

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 238.01/U26

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH R01AA026186
Brain and Behavior Research Foundation

Title: Diacylglycerol lipase a therapeutic target for alcohol use disorder

Authors: *G. BEDSE[1], S. E. YOHN[2], A. ASTAFYEV[2], T. A. PATRICK[2], J. UDDIN[2], P. CONN[2], L. J. MARNETT[2], S. PATEL[2];
[1]Psychiatry, Vanderbilt Univ. Med. Ctr., Nashville, TN; [2]Vanderbilt Univ., Nashville, TN

Abstract: Alcohol use disorder (AUD) is a severe form of problematic drinking characterized by compulsive and uncontrollable alcohol use. Currently available treatment options do not adequately address this human health problem and thus, novel treatments are desperately needed. Recent preclinical studies have indicated the role of endocannabinoid system in the behavioral and physiological effects of alcohol, particularly in the alcohol seeking behavior. However, the role of 2-arachidonoyl glycerol (2-AG) signaling in alcohol drinking is still unclear. In this study, we utilized pharmacological, genetic, neuronal circuit manipulation and in-vivo fast scanning cyclic voltammetry techniques to demonstrate a critical role for the 2-AG in ethanol drinking and diacylglycerol lipase (DAGL), 2-AG synthesizing enzyme, could be a novel therapeutic target for AUD.

C57BL/6j mice, subjected to 6 weeks of chronic continuous access in a two-bottle choice paradigm, developed high ethanol intake and preference. We showed that genetic deletion of diacylglycerol lipase α (DAGLα), in the central nervous system, robustly reduced alcohol preference and consumption in a sex-specific manner. We further showed that systemic administration of DAGL inhibitor, DO34 (50 mg kg^{-1}, 3 daily injections), robustly reduced alcohol preference and consumption in mice. However, pharmacological augmentation of 2-AG did not increase alcohol preference and consumption. We investigated potential side effects of DO34 in open field, social interaction, elevated zero maze (EZM), tail suspension test (TST) and light-dark box assay. DO34 treatment did not show any side effects in aforementioned behavioral tests. In contrary, DO34 treatment decreased anxiety- and depressive-like behaviours in EZM and TST, respectively. The effects of DAGL inhibition are specific to alcohol drinking, as systemic administration of DO34 (50 mg kg^{-1}), did not reduce sucrose preference. To understand the brain region specific role of 2-AG, we deleted DAGLα in the nucleus accumbens (NAc), a key brain region in the alcohol reward system. NAc-specific DAGL deletion decreases ethanol consumption in the female mice but not in male mice, suggesting important role of 2-AG in the alcohol reward. By using in vivo fast scanning cyclic voltammetry (FSCV), we tested effects of DO34 treatment on the NAc dopamine release. Acute systemic ethanol administration increased DA release and DO34 treatment attenuated ethanol-induced increase in DA release in NAc. These data suggest 2-AG plays an important role in alcohol drinking and that pharmacological inhibition of DAGLα could represent a novel approach for AUD treatment.

Disclosures: G. Bedse: None. **S.E. Yohn:** None. **A. Astafyev:** None. **T.A. Patrick:** None. **J. Uddin:** None. **P. Conn:** None. **L.J. Marnett:** None. **S. Patel:** None.

Poster

238. Addiction Treatment

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 238.02/U27

Topic: G.08. Drugs of Abuse and Addiction

Support: PUT1686

Title: The effect of cocaine and amphetamine treatment on DNA methylation and demethylation in human peripheral blood mononuclear cells

Authors: *K. ANIER, M. URB, M. ALTTOA, K. SIKK, K. SOMELAR, A. KALDA;
Univ. of Tartu, Tartu, Estonia

Abstract: An increasing number of reports have provided crucial evidence that epigenetic modifications, such as DNA methylation and demethylation, may be involved in initiating and establishing psychostimulant-induced stable changes at the cellular level by coordinating the expression of gene networks, which then manifests as long-term behavioral changes. Recent discoveries suggest that ten-eleven translocation enzymes (TET1, TET2, TET3) participate in the DNA demethylation process and might also play a role in the action of psychostimulants. Repeated cocaine administration downregulates TET1 in *nucleus accumbens* (NAc), alters 5-hydroxymethylcytosine (5-hmC) distribution and the expression of differentially spliced isoforms (Feng et al., 2015). Given the difficulty of obtaining human brain tissue, a key question in molecular psychiatry concerns the extent to which epigenetic signatures measured in more accessible tissues, such as peripheral blood mononuclear cells (PBMCs), can serve as a surrogate marker for the brain.

Our previous study demonstrated that cocaine can modify gene transcription, via DNA methylation and demethylation, throughout the brain and as well as in peripheral blood cells (Anier et al., 2018). Therefore, the aim of this study was to investigate the effect of cocaine and amphetamine treatment on both DNA methylation and demethylation in the human PBMCs.

In this *in vitro* study, we evaluated an acute effect of cocaine or amphetamine treatment on *Tet1-3* and DNA methyltransferases' *Dnmt1*, *Dnmt3a*, *Dnmt3b* expression in the human PBMCs. PBMCs from healthy donors (male, age 20-35) were treated with amphetamine (0.3 mg/l) or cocaine (3 mg/l) for 0.5; 1; 1.5; 2; 4 and 24 hours. Our qPCR data showed that both cocaine and amphetamine dynamically downregulates *Tet1-3* mRNA after acute treatment in PBMC. Repeated cocaine (3 mg/l) or amphetamine (0.3 mg/l) treatment for 4 consecutive days significantly reduced *Tet1-3* mRNA levels in PBMCs. These data are in line with TET enzyme activity results, which also demonstrated that both repeated cocaine and amphetamine treatment significantly decreased TET activity in PBMCs. We also found that repeated cocaine or amphetamine treatment for 4 days upregulates *Dnmt1* mRNA, but did not affect DNMT activity in PBMCs.

Based on this data, we conclude that cocaine and amphetamine treatment may disturb the equilibrium between DNA methylation and demethylation processes in PBMCs.

Disclosures: K. Anier: None. **M. Urb:** None. **M. Alttoa:** None. **K. Sikk:** None. **K. Somelar:** None. **A. Kalda:** None.

Poster

238. Addiction Treatment

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 238.03/U28

Topic: G.08. Drugs of Abuse and Addiction

Support: R00 AA023559

Title: Nociceptin attenuates alcohol drinking in a sex dependent manner

Authors: *P. GHAZAL, K. PLEIL;
Dept. of Pharmacol., Weil Cornell Med. Col., Newyork, NY

Abstract: Alcohol use disorder is one of the most prevalent neuropsychiatric diseases globally, with enormous socio-economic and health consequences. While alcohol addiction is more prevalent in males, this gender gap is closing and women suffer greater biological and psychological consequences of alcohol abuse than men, as well as telescoping behavior (more rapid development of addiction from first use). Accumulating evidence show that the sex hormone estrogen enhances the rewarding properties of drugs and promotes drug-seeking behaviors in a fluctuating manner across the menstrual cycle. Further, clinical studies have repeatedly shown that treatments available for alcohol addiction like Naltrexone are less efficacious for female alcoholics than males, potentially due to the drug reward-promoting actions of estrogen. Nociceptin (NOP) is an opioid neuropeptide that acts through its primary receptor ORL-1 to decrease excessive alcohol use and prevent addiction and/or relapse in preclinical models of AUD in males. Here we evaluated the ability of NOP to modulate alcohol consumption in females and examined whether estrogen blunts the anti-drinking effects of NOP using the Drinking in the Dark (DID) binge drinking paradigm. We found that an ORL-1 agonist administered systemically was more effective in reducing binge drinking in males than intact female mice, and ongoing experiments are determining whether this is estrous cycle stage-dependent in females. We are also investigating the site(s) of NOP-estrogen interactions in the brain that underlie behavioral effects and mechanisms of these interactions. Our studies may provide mechanistic insight that contributes to the development of novel pharmacological strategies for the treatment of alcohol abuse and addiction in females.

Disclosures: P. Ghazal: None. **K. Pleil:** None.

Poster

238. Addiction Treatment

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 238.04/U29

Topic: G.08. Drugs of Abuse and Addiction

Support: The Brain Research Foundation

Title: Intranasal glial cell line-derived neurotrophic factor gene therapy for opioid use disorder

Authors: M. J. KYADA[1], S. IRIAH[1], O. SESENOGLU-LAIRD[2], L. PADEGIMAS[3], M. J. COOPER[2], *B. L. WASZCZAK[1];
[1]Pharmaceut. Sci., Northeastern Univ., Boston, MA; [2]Copernicus Therapeutics, Inc., Cleveland, OH; [3]Abeona Therapeutics, Inc., Cleveland, OH

Abstract: Glial cell line-derived neurotrophic factor (GDNF) is a growth-promoting protein that supports the survival and function of brain dopamine neurons, including those which mediate drug reward. Previously, GDNF was shown to have promise as a treatment for drug addiction, especially opioid use disorder (OUD). We hypothesize that a GDNF gene therapy could prevent and/or correct the dopamine deficiency state and the reward deficit that develop during the chronic use of opioids. To test this hypothesis, we investigated whether intranasal delivery of plasmid DNA nanoparticles (NPs) generating GDNF within the brain could reduce the development, persistence, and/or relapse to oxycodone (OXY) in rats. We used a validated drug reward paradigm, conditioned place preference (CPP), in which rats received 3 mg/kg OXY or saline on alternating days for 8 days and then were tested for their preference for the OXY-paired versus the saline-paired side of the conditioning chamber. We evaluated whether intranasal pGDNF NPs could: 1) reduce the initial development of CPP to OXY (reduce reward), 2) decrease the persistence of CPP over a period of 14 days extinction, and 3) suppress reinstatement of OXY-induced CPP after extinction. When given before OXY training, intranasal pGDNF NPs did not significantly alter the magnitude of OXY-induced CPP compared to rats given intranasal saline, suggesting no change in the initial reinforcing effects of opioids. However, results of the second study showed a significant reduction in the persistence of OXY CPP beginning 1 week after intranasal administration of pGDNF NPs, but no decrease in CPP in rats given intranasal saline. Interim results of the third study showed that intranasal pGDNF NPs given after 14 days extinction significantly reduced reinstatement of CPP to a priming dose of OXY (1.5 mg/kg) 7 days later, compared to rats given intranasal saline. These results are consistent with our hypothesis that increasing brain GDNF levels after discontinuation of opioid drug use may suppress drug craving and reduce relapse potential by promoting recovery of the mesolimbic dopamine system, leading to normalized dopamine levels and reward system function. Ultimately, intranasal pGDNF NPs could become a novel, non-invasive, long-lasting

form of gene therapy for relieving craving and reducing relapse in patients with OUD attempting to discontinue use of opioids.

Disclosures: **M.J. Kyada:** None. **S. Iriah:** None. **O. Sesenoglu-Laird:** A. Employment/Salary (full or part-time):; Copernicus Therapeutics, Inc. **L. Padegimas:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Copernicus Therapeutics, Inc. **M.J. Cooper:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Copernicus Therapeutics, Inc.. **B.L. Waszczak:** None.

Poster

238. Addiction Treatment

Location: Hall A

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Program #/Poster #: 238.05/U30

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH grant R01 AA019793
NIH T32 training grant

Title: Effects of a CRFR1 antagonist on alcohol drinking and affiliative behavior in prairie voles

Authors: *S. POTRETZKE, M. ROBINS, A. RYABININ;
Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: The prevalence and detrimental consequences of alcohol misuse necessitate the development of efficacious treatments. The corticotropin-releasing factor (CRF) system has been implied as a promising target with demonstrated effects on alcohol consumption in both dependence-induced drinking and binge-like consumption paradigms. However, while CRF1 receptor (CRFR1) antagonists have been shown to be effective in decreasing alcohol consumption in preclinical models, these effects have failed to translate clinically. We hypothesized this discrepancy may stem from known differences in the social behaviors and CRF system of the traditional laboratory animals (i.e. mice and rats) and humans. To address this hypothesis, we compared the effects of a CRFR1 antagonist on alcohol consumption in the socially monogamous prairie voles (*Microtus ochrogaster*)- a rodent species with demonstrated translational validity of social mechanisms to humans- and C57BL/6J mice. Individually housed male and female animals underwent continuous access two-bottle (alcohol and water) choice procedures. Following a 1-week drinking procedure with increasing concentrations of alcohol, with 2 days of baseline consumption of a 10% alcohol (v/v water), animals received intraperitoneal (IP) injections of saline or a selective CRFR1 antagonist, CP-376395 (1mg/kg, 3mg/kg, 10mg/kg or 20mg/kg). Alcohol consumption and preference ratio were determined over the first 3 hours. We observed a trend for decreased alcohol consumption and preference at the

two highest doses. In mice, CP-376395 significantly decreased both alcohol consumption and preference at these doses. Our ongoing experiments test the effects of the CRFR1 on partner preference in prairie voles. We hypothesize that the contrast observed between the effects of the CRFR1 antagonist in prairie voles and mice could reflect the difference in distribution of CRFR1 between monogamous vole species and other rodents. CRFR1 binding in the nucleus accumbens is lower in prairie voles than other rodent species. Humans also show low CRFR1 receptor expression in the nucleus accumbens. Together, these results provide further evidence that socially monogamous rodent species may serve as better animal models to test potential pharmacotherapies for excessive alcohol use and other mental disorders.

Disclosures: S. Potretzke: None. **M. Robins:** None. **A. Ryabinin:** None.

Poster

238. Addiction Treatment

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 238.06/U31

Topic: G.08. Drugs of Abuse and Addiction

Support: CONACYT-FOSISS project No. 0260971

Title: Changes in resting state networks in cocaine addicts after rTMS treatment on the left DLPFC

Authors: S. FERNANDEZ[1], R. ALCALA-LOZANO[2], S. ALCAUTER[3], J. J. GONZÁLEZ-OLVERA[4], ***E. A. GARZA-VILLARREAL**[1];
[1]LANIREM, Inst. of Neurobiology, Univ. Nacional Autonoma de Mexico (UNAM), Queretaro, Mexico; [2]Clin. Res. Div., Inst. Nacional de Psiquiatria, Mexico City, Mexico; [3]Behavioral and Cognitive Neurobiolgy, Inst. De Neurobiologia. Univ. Nacional Autonoma de Mexico, Queretaro, Mexico; [4]INSTITUTO NACIONAL DE PSIQUIATRIA, Mexico City, Mexico

Abstract: There is a lack of effective treatments for drug addiction (about 40% to 60% relapse) (McLellan et al. 2000). In the present clinical trial, we evaluated the effect of repetitive transcranial magnetic stimulation (rTMS) on craving and impulsivity in cocaine addicts. We recruited current cocaine users with DSM-5 diagnosis of cocaine addiction. The patients were randomly and double-blindly assigned to a sham or real- rTMS group and treated for 2 weeks (acute phase or close-label) with 5 Hz rTMS on the left DLPFC. After the acute phase they received weekly maintenance sessions for 3 months as open-label. Clinical and neuroimage data were acquired after every stage of the study. As clinical outcomes we considered: 1) craving (VAS) and 2) impulsivity (BIS-11). Neuroimaging data was acquired on a 3T Philips Ingenia scanner and a 32-channel coil. We acquired structural T1w images and rsfMRI for 10 min and performed preprocessing using several tools. For graph theory analysis, we used a consensus-

based thresholding (de Reus and van den Heuvel 2013) (t≥.2; 65%) to: 1) create weighted and undirected graphs of the whole brain (WB) and seven networks related to craving and executive processes (CON, DAN, DMN, FPN, SAL, SUB and VAN), and 2) explore their cost (strength, S, and density, D), efficiency (local, EL, and global, EG) and small-worldness (SW, calculated from the mean clustering coefficient, CP, and characteristic path length, LP) (Maslov and Sneppen 2002). Appropriate GLMs were used and FDR-corrected alpha of .05 was used. Finally, we correlated the changes of graph metrics and clinical data. Impulsivity and craving decreased significantly after real-rTMS over sham (impulsivity, $F(1,25)=8.98$, $p=.006$) (craving, $F(1,25)=3.90$, $p=.05$). We found changes both in the global network and most of the subnetworks in all stages. CON, SUB and VAN networks showed an increase in cost ($p<.001$) and efficiency ($p<.001$) with real-rTMS compared to sham, and higher efficiency ($p<.001$), cost (CON and SUB, $p<.001$) and clustering (CON and VAN, $p<.001$) after two weeks. Local efficiency of SUB decreased after maintenance ($p<.001$). These same networks' changes in cost and efficiency were also related to the changes in impulsivity. This study provides evidence of rTMS as an effective treatment in addiction due to its effects on both impulsivity and craving and on brain connectivity networks topology, however its long-lasting effectivity is something that needs to be explored further. Furthermore, these results provide insight regarding graph theory analysis as a suitable tool to assess both the nature of addiction and the efficacy of its treatments.

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Poster

238. Addiction Treatment

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 238.07/U32

Topic: G.08. Drugs of Abuse and Addiction

Title: Effect of lorcaserin and atomoxetine in two tests of compulsive action

Authors: *G. A. HIGGINS, M. BROWN, C. MACMILLAN, L. B. SILENIEKS; InterVivo Solutions Inc, Toronto, ON, Canada

Abstract: Although the 5-HT_{2C} receptor agonist lorcaserin (LOR) has been approved for obesity, there is a view that this drug may be effective in other eating disorders, such as binge eating disorder (BED). The selective noradrenergic reuptake inhibitor atomoxetine (ATX), approved for ADHD, has also been considered as a treatment for BED. Both LOR and ATX are effective in reducing behaviours related to impulsive action, which may contribute to effective treatment of BED. The present investigations were designed to examine both drugs in two tests of compulsive action - schedule-induced polydipsia (SIP) and dizocilpine (DZP)-induced increase in perseverative (PSV) responding in the 5-choice task (5-CSRTT). Male, Wistar rats

were given access to intermittent food (available under an FI60s schedule) with concomitant access to water. A sub-population of rats consumed a significant volume of water (>15mL) during the 1h session despite not being water deprived, i.e SIP. Pretreatment with LOR (0.1-0.6 mg/kg SC) and ATX (0.1-1 mg/kg IP) reduced water intake during food access, suggestive of a blunting of this measure of compulsive action (e.g. Veh: 19.8±3.3mL; LOR 0.6 mg/kg: 10.7±1.8mL; $P<0.05$). Control experiments confirmed selectivity of each drug against this compulsive behaviour. In male Long Evans rats trained to stable 5-CSRTT performance (0.75s SD; 5s ITI; 100 trials), dizocilpine (0.01-0.06 mg/kg SC) produced robust increases in PSV responses (e.g PSV responses: Veh: 24±3, DZP 0.03 mg/kg: 65±16; $P<0.01$). Further analysis suggested that the majority of PSV responses increased by dizocilpine were in the previously correct niche. Pretreatment with LOR (0.3-0.6 mg/kg SC) and ATX (0.5-1 mg/kg IP) blunted the increased PSV responses induced by dizocilpine (0.03 mg/kg). The present studies confirm a reliable effect of LOR and ATX to attenuate two measures of compulsive action. Together with the robust effects of both drugs against measures of impulsive action, these properties may contribute to the view that both drugs should be considered for certain eating disorders such as BED, as well as conditions characterized by addiction.

Disclosures: G.A. Higgins: None. **M. Brown:** None. **C. MacMillan:** None. **L.B. Silenieks:** None.

Poster

238. Addiction Treatment

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant HHSN271201800035C

Title: Portable transcranial direct current stimulation (tDCS) to reduce craving in cocaine addiction: A double-blind sham-controlled phase 1 clinical trial

Authors: *P.-O. GAUDREAULT[1], A. DATTA[2], P. MALAKER[1], A. WAGNER[1], M. A. PARVAZ[1], E. NAKAMURA-PALACIOS[3], L. C. PARRA[4], N. ALIA-KLEIN[1], R. Z. GOLDSTEIN[1];
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Abstract: Drug addiction is a chronic brain disorder with the highest propensity to relapse in the first year of abstinence.[1] Drug craving, the intense subjective urge to use drugs, predisposes drug addicted individuals to relapse and predicts clinical outcomes.[2] However, reducing craving in

drug addiction remains a challenge. The prefrontal cortex (PFC), and more specifically the dorsolateral PFC (DLPFC), has been identified as a key structure in the drug addiction cycle, associated with disruption of inhibitory control and salience attribution.[3] A recent study demonstrated significant reduction in craving after 5 sessions of transcranial direct current stimulation (tDCS) over the DLPFC in 36 outpatient individuals with cocaine use disorder (iCUD) in Brazil.[4] The goal of the current phase 1 clinical trial is to replicate these previous findings in a northeastern US urban population of treatment-seeking iCUD while validating the ultimate home use of a portable tDCS device. For this pilot study, 15 abstinent iCUD are being recruited from an inpatient drug addiction treatment facility and randomly assigned to either an active-tDCS (2mA, 35 cm^2, left cathodal/right anodal) or sham-tDCS condition (control group). Each subject will undergo 15 sessions of tDCS (20 min) every other day over five weeks. Self-reported cocaine craving will serve as the primary clinical outcome. Secondary outcomes include levels of depression, anxiety, and quality of life as well as implicit verbal measures of drug cue reactivity. Data is currently being acquired in a double-blind manner (9 subjects screened; 4 started stimulations; all subjects/sessions to be completed and data analyzed before the SfN meeting). We predict a decrease in craving (and in other cue-reactivity measures) after active-tDCS as compared to the sham-tDCS. Specifically, we predict a group by time (pre- vs post-treatment) interaction to be explained by a more pronounced decrease in craving in the former vs the latter group. The putative mechanism encompasses enhancing DLPFC functions including improvements in working memory but also other higher-order executive functions (e.g., self-control, salience attribution and awareness), as potentially indicative of enhanced function in interconnected regions (e.g., ventromedial PFC), as remains to be tested. In future extensions of this pilot study, we plan to increase the number of subjects and include neuroimaging scans as well as cognitive and behavioral measures to more directly identify the underlying mechanisms of tDCS effect on reducing craving in cocaine addiction. References: [1] Dennis et al., 2007 [2] Carter & Tiffany, 1999 [3] Goldstein & Volkow, 2011 [4] Batista et al. 2015

Disclosures: **P. Gaudreault:** None. **A. Datta:** None. **P. Malaker:** None. **A. Wagner:** None. **M.A. Parvaz:** None. **E. Nakamura-Palacios:** None. **L.C. Parra:** None. **N. Alia-Klein:** None. **R.Z. Goldstein:** None.

Poster

238. Addiction Treatment

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 238.09/U34

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Intramural Funding ZIA-AA000218 (PI: Lorenzo Leggio), jointly supported by NIAAA Division of Intramural Clinical and Biological Research and NIDA Intramural Research Program

NCATS Grant UH2/UH3-TR000963 (PIs: Lorenzo Leggio and Fatemeh Akhlaghi)
Development of the Computerized Alcohol Infusion System (CAIS) supported by the NIAAA-funded Indiana Alcohol Research Center (AA007611)
Pfizer provided the PF-5190457 drug under the grant UH2/UH3-TR000963 grant

Title: Ghrelin: From a gut hormone to a potential therapeutic target for alcohol addiction

Authors: *V. MUNJAL[1], M. FAROKHNIA[1,2], M. R. LEE[1], M. L. SCHWANDT[3], L. A. FARINELLI[1], F. AKHLAGHI[4], L. LEGGIO[1,2,5,6];
[1]NIAAA/NIDA, [2]Ctr. on Compulsive Behaviors, [3]NIAAA, NIH, Bethesda, MD; [4]Clin. Pharmacokinetics Res. Lab., Univ. of Rhode Island, Col. of Pharm., Kingston, RI; [5]Medication Develop. Program, Natl. Inst. on Drug Abuse, Baltimore, MD; [6]Ctr. for Alcohol and Addiction Studies, Brown Univ., Providence, RI

Abstract: Alcohol use disorder (AUD) is a chronic relapsing disease with medical, psychosocial, and economic burdens. Pharmacological treatments for AUD are, however, limited in number and efficacy. Beyond classic neurotransmitter systems, there has been a growing interest in understanding the role of peripheral pathways (e.g., gut-brain communications) in AUD, with the final goal of identifying novel therapeutic targets. Ghrelin is an orexigenic peptide synthesized by enteroendocrine cells primarily located in the gastric mucosa. Ghrelin is known as the 'hunger hormone', given its role in stimulating both homeostatic and hedonic food intake. Ghrelin has also been shown to modulate alcohol seeking and consummatory behaviors, possibly through interactions with reward and stress pathways. Here we investigated whether and how pharmacological manipulation of the ghrelin system may affect biobehavioral correlates of alcohol use in heavy-drinking alcohol-dependent individuals. First, in a randomized, double-blind, placebo-controlled, human laboratory study, intravenous (IV) ghrelin/placebo was administered and two experiments were conducted: progressive-ratio IV alcohol self-administration (IV-ASA) and brain functional magnetic resonance imaging (fMRI). Results showed that IV ghrelin, compared to placebo, significantly increased the number of alcohol infusions self-administered (p=0.04) and reduced the time to initiate alcohol self-administration (p=0.03). In addition, ghrelin increased the alcohol-related signal in the amygdala (p=0.01) and modulated the food-related signal in the medial orbitofrontal cortex (p=0.01) and nucleus accumbens (p=0.08). In the second study, we examined the effects of a novel ghrelin receptor inverse agonist (PF-5190457) co-administered with alcohol in both rodents and humans. No pharmacological interactions between alcohol and PF-5190457 were found. PF-5190457 did not interact with the effects of alcohol on locomotor activity or loss-of-righting reflex (p's>0.05). In humans, PF-5190457 was well tolerated, did not cause any significant side effect, and did not alter alcohol pharmacokinetics or alcohol-induced subjective effects (p's>0.05). As a secondary outcome, a cue-reactivity procedure was also conducted in a bar-like laboratory setting. Results showed that PF-5190457, compared to placebo, reduced cue-elicited craving for alcohol (p=0.05), as well as attention to alcohol cues (p=0.02). In conclusion, the ghrelin system seems to regulate both behavioral and neurobiological correlates of alcohol consumption and may represent a potential target for developing novel medications to treat AUD.

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Poster

238. Addiction Treatment

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 238.10/U35

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R01 DA015214
NIH Grant T32 DA028874

Title: Deep brain stimulation suppresses cocaine-seeking behavior by selectively depotentiating accumbens shell D2DR-containing neurons

Authors: ***M. T. RICH**, S. E. SWINFORD-JACKSON, P. J. HUFFMAN, R. C. PIERCE; Univ. of Pennsylvania, Philadelphia, PA

Abstract: Despite decades of research, cocaine addiction remains a serious public health concern, as there are no FDA-approved treatments for the prevention of relapse. The use of deep brain stimulation (DBS) for the treatment of neurological disorders has steadily increased in recent years and has been proposed as a potential therapy for the suppression of craving and relapse. However, the cellular and physiological mechanisms underlying the efficacy of DBS remain unknown. Medium spiny neurons (MSNs) are the major projection neurons of the nucleus accumbens (NAc) and are subdivided into two classes based on the expression of either dopamine D1 receptors (D1DRs) or dopamine D2 receptors (D2DRs). Canonically, activation of D1DR-containing MSNs promotes, while activation of D2DR-containing MSNs inhibits, cocaine-seeking behavior. Previous work from our lab suggests that DBS of the NAc shell reduces cocaine-primed reinstatement in rats across a broad range of frequencies (12-130 Hz). Subsequent studies using optogenetic stimulation to mimic DBS indicates that the relapse-inhibiting behavioral effect is driven specifically by actions at D2DR-containing MSNs. This cellular and behavioral divergence suggests that DBS-induced suppression of cocaine reinstatement may be mediated by differential effects at the two MSN subtypes. To investigate the mechanisms of DBS, we performed whole-cell patch clamp recordings from NAc shell MSNs. Electrical stimulation (12 Hz) of NAc afferents evoked oppositional LTD- or LTP-like responses in individual neurons through changes in presynaptic signaling, suggesting that DBS may differentially affect plasticity at D1DR- and D2DR-containing MSNs. To determine if depotentiation occurs specifically at D2DR-containing neurons, ongoing electrophysiological studies are utilizing transgenic rat lines that selectively express Cre recombinase in D2DR-containing neurons in combination with a Cre-dependent adeno-associated viral vector expressing channelrhodopsin (ChR2-eYFP). Initial data indicates that optogenetic DBS-like

stimulation (12 Hz) at D2DR-containing MSNs does indeed promote LTD. Taken together, our results show that DBS-induced attenuation of cocaine-seeking behavior is dependent on synaptic remodeling within the NAc shell, and likely involves depotentiation specifically at D2DR-containing MSNs.

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Poster

238. Addiction Treatment

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 238.11/U36

Topic: G.08. Drugs of Abuse and Addiction

Support: R41 DA047169-01

Title: Troriluzole reduces development, and enhances extinction, of oxycodone conditioned place preference and attenuates cocaine relapse and locomotor sensitization in rats

Authors: V. W. LI[1], S. NAYAK[2], T. JENNINGS[2], S. GAO[1], V. DUSZAK[1], G. NEALE[1], M. WATSON[2], A. B. REITZ[3], R. W. SCOTT[3], K. M. FREEMAN[3], A. SHAIKH[1], Z. STUBLAREC[1], S. LOPEZ[1], A. WARREN[1], ***J. A. SCHROEDER**[1], S. M. RAWLS[2]; [1]Behavioral Neurosci. Program, Connecticut Col., New London, CT; [2]Lewis Katz Sch. of Medicine, Temple University, Ctr. for Substance Abuse Res., Philadelphia, PA; [3]Fox Chase Chem. Diversity Ctr., Doylestown, PA

Abstract: Chronic psychostimulant and opioid exposure results in increased glutamatergic tone in the nucleus accumbens which has been implicated as a contributing factor in addiction to both drug classes. The glutamate transporter subtype 1 (GLT-1) activators ceftriaxone and clavulanic acid attenuate psychostimulant and opioid place preference and self-administration. Riluzole, an approved medication for amyotrophic lateral sclerosis, reduces cocaine relapse in rat models of self-administration but, from a translational perspective, suffers from a host of pharmacokinetic and metabolic limitations, which include high first-pass hepatic metabolism, elevation of liver enzymes, a negative food effect, low aqueous solubility, and poor oral palatability. Troriluzole (TRLZ), a third generation prodrug of riluzole representing >5 years of chemistry effort taken from >400 potential analogs, displays optimized pharmacokinetic and metabolic features and is currently being tested in clinical trials for obsessive compulsive disorder and spinal cerebellar ataxia. The current study examined the effects of TRLZ on reward- and relapse-related behaviors of oxycodone and cocaine in rats. TRLZ administered concurrently with oxycodone significantly reduced the development of oxycodone place preference, and when administered following oxycodone conditioning, enhanced oxycodone place preference. In rats trained to self-administer

cocaine under fixed-ratio conditions, the administration of TRLZ during the subsequent extinction phase significantly reduced cue-induced cocaine seeking behaviors. In locomotor experiments, TRLZ reduced behavioral sensitization produced by repeated cocaine exposure. These preclinical data showing that TRLZ reduces opioid reward and attenuates cocaine relapse and locomotor sensitization suggest efficacy against multiple classes of addictive drugs. Because TRLZ reduces glutamate transmission through a dual mechanism of action, it may offer therapeutic advantages compared to β-lactam drugs that primarily target glutamate transport systems.

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Poster

238. Addiction Treatment

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 238.12/U37

Topic: G.08. Drugs of Abuse and Addiction

Support: This work was supported by NIDA/NIH.

Title: Sex-specific effects of the monoamine stabilizer (-)-OSU6162 on relapse of oxycodone seeking after electric barrier-induced voluntary abstinence

Authors: *S. V. APPLEBEY, J. M. BOSSERT, Y. SHAHAM, I. FREDRIKSSON; Behavioral Neurosci. Br., IRP-NIDA, NIH., Baltimore, MD

Abstract: Since the late 1990s, prescription opioid addiction and overdose rates have dramatically increased in the United States, underscoring a need for new pharmacological treatments. High relapse rates are likely a major contributor to this crisis. In humans, abstinence is often motivated by a desire to avoid adverse consequences. However, most rat relapse models use forced abstinence. Here we examined relapse after abstinence due to adverse consequences and tested whether the monoamine stabilizer (-)-OSU6162 would decrease oxycodone self-administration and relapse. We first trained male and female rats to self-administer oxycodone (0.1 mg/kg/infusion, 6-h/d) for 14 days. We then achieved voluntary abstinence by introducing an electric barrier of increasing intensity (0.1 to 0.4 mA) near the drug-paired lever. On abstinence day 15, we tested the rats for relapse to oxycodone seeking in absence of drug and barrier after either vehicle or OSU6162 (7.5 or 15 mg/kg, s.c.) injections. Next, we retrained rats (n=6-7/sex) to self-administer oxycodone (0.1 mg/kg/infusion, 6-h/d) under both fixed (FR-1) and progressive ratio reinforcement schedules and tested the effect of vehicle or (-)-OSU6162

(7.5 or 15 mg/kg) on oxycodone self-administration. In male but not female rats, (-)-OSU6162 decreased relapse of oxycodone seeking after electric barrier-induced voluntary abstinence. In contrast, (-)-OSU6162 had no effect on ongoing oxycodone self-administration in males or females under either reinforcement schedule. Together, these data demonstrate sex-specific effects of (-)-OSU6162 on relapse after voluntary abstinence due to adverse consequences of drug-seeking, suggesting that (-)-OSU6162 should be considered for relapse prevention in men addicted to opioids.

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Poster

238. Addiction Treatment

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 238.13/U38

Topic: G.08. Drugs of Abuse and Addiction

Support: IRP/NIDA/NIH

Title: Opioid maintenance delivery of the biased agonist TRV130 decreases relapse to oxycodone seeking and prevents acute opioid-induced brain hypoxia

Authors: *J. M. BOSSERT[1], E. KIYATKIN[1], H. KORAH[1], J. K. HOOTS[1], I. FREDRIKSSON[1], B. E. BLOUGH[2], Y. SHAHAM[1];
[1]IRP/NIDA/NIH, Baltimore, MD; [2]Ctr. for Drug Discovery, Res. Triangle Inst., Research Triangle Park, NC

Abstract: Background: High relapse rate is a major obstacle in addressing the current opioid crisis. Opioid agonist maintenance therapy (buprenorphine, methadone) is an effective treatment for opioid addiction, but does not prevent relapse in all opioid users. We modified the context-induced reinstatement model in rats trained to self-administer the prescription opioid oxycodone and compared the efficacy of buprenorphine with that of the novel opioid mu opioid receptor (MOR) biased agonist TRV130. Methods: We trained rats to self-administer oxycodone (6-h/d, 14 d) in context A; infusions were paired with a discrete tone-light cue. We then implanted Alzet osmotic pumps containing vehicle or 3, 6, or 9 mg/kg/d of buprenorphine or TRV130 and performed three relapse-related tests: (1) responding for oxycodone-paired discrete cues under extinction conditions in a non-drug context (context B), (2) context-induced reinstatement of oxycodone seeking in context A, and (3) reacquisition of oxycodone self-administration in context A. We also assessed the effect of chronic TRV130 delivery on acute oxycodone-induced decreases in oxygen levels in nucleus accumbens. Results: Chronic buprenorphine delivery significantly decreased cue-induced oxycodone seeking in context B and reacquisition of

oxycodone self-administration in context A. Chronic delivery of TRV130 significantly decreased relapse-related behaviors on all three measures. Chronic TRV130 delivery also prevented acute oxycodone-induced brain hypoxia. Conclusions: Chronic delivery of TRV130 decreased oxycodone seeking on multiple measures of relapse and prevented acute opioid-induced brain hypoxia. We propose that biased MORs agonists, currently developed for pain treatment, should also be considered as opioid agonist maintenance treatment for opioid addiction.

Disclosures: **J.M. Bossert:** None. **E. Kiyatkin:** None. **H. Korah:** None. **J.K. Hoots:** None. **I. Fredriksson:** None. **B.E. Blough:** None. **Y. Shaham:** None.

Poster

238. Addiction Treatment

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 238.14/U39

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH intramural funds (Yavin Shaham)
1FI2GM128603 (David Reiner)

Title: Role of orbitofrontal cortex in relapse to fentanyl seeking after food-choice induced voluntary abstinence

Authors: *D. J. REINER[1], O. M. LOFARO[1], M. VENNIRO[1], C. CIFANI[2], J. M. BOSSERT[1], Y. SHAHAM[1];
[1]IRP-NIDA, NIH, Baltimore, MD; [2]Univ. of Camerino, Sch. of Pharm., Camerino, Italy

Abstract: There are few preclinical studies of fentanyl relapse, and these studies have used experimenter-imposed (forced) abstinence procedures. In humans, however, abstinence is often voluntary, with drug available in the drug environment but forgone in favor of non-drug alternative rewards. We recently developed a rat model of relapse after food choice-induced voluntary abstinence. Here we used the model to study the role of orbitofrontal cortex (OFC), previously implicated in heroin relapse after forced abstinence, in relapse to fentanyl seeking after voluntary abstinence. We trained male and female rats to self-administer palatable food pellets for 6 days (6-h/day) and fentanyl (2.5 microgram/kg/infusion, i.v.) for 12 days (6-h/day). We assessed relapse to fentanyl seeking after 14 voluntary abstinence days, achieved through a discrete choice procedure between fentanyl and palatable food (20 trials/day). In both sexes, relapse was associated with increased expression of the activity marker Fos in lateral and ventral orbitofrontal cortex (OFC). Reversible inactivation of either subregion with GABA-A and GABA-B receptor agonists muscimol and baclofen (50+50 ng/side) decreased relapse to fentanyl seeking. We next determined projection-specific activation of OFC afferents during the relapse test by using Fos plus the retrograde tracer cholera toxin B (injected into OFC). Relapse to

fentanyl seeking was associated with increased Fos expression in piriform cortex neurons projecting to OFC, but not OFC-projecting basolateral amygdala or thalamic neurons. Results demonstrate a role of OFC in relapse to fentanyl seeking after voluntary abstinence and suggest a potential role of the projection from piriform cortex to OFC in this relapse.

Disclosures: D.J. Reiner: None. **O.M. Lofaro:** None. **M. Venniro:** None. **C. Cifani:** None. **J.M. Bossert:** None. **Y. Shaham:** None.

Poster

238. Addiction Treatment

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 238.15/U40

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA U01DA047713
BRINM

Title: Targeting PTPRD D1 phosphatase: Message and address?

Authors: *G. R. UHL[1], D. A. JOHNSON[2], W. WANG[3], T. PRISINZANO[4];
[1]Neurology, Reserach, Neuroscience, Mol. Genet. & Microbiology, Univ. of New Mexico and NMVAHCS, Albuquerque, NM; [2]Pharmacol., Univ. of Kansas, Lawrence, KS; [3]Col. of Pharm., Univ. of Arizona, Tucson, AZ; [4]Col. of Pharm., Univ. of Kentucky, Lexington, KY

Abstract: PTPRD is a receptor type protein tyrosine phosphatase that is expressed interesting neurons, providing a prominent phosphatases in cortical synaptosomes. PTPRD genomic variants have been associated with individual differences in vulnerability to addictions, ability to quit smoking, reward from amphetamine administration and levels of expression of PTPRD mRNA in postmortem brains. Mice with altered levels of PTPRD expression display marked differences in cocaine-conditioned place preference and self administration as well as disruption of sleep measured in the two hours around lights on. While each of these features increases interest in drugs that could modify PTPRD activities, there has been no documented ligand for PTPRD. We have identified an illudalic acid analog, 7-BIA, that was identified as active at a related phosphatase, inhibits activity of recombinant human PTPRD phosphatase domain fusion protein with micromolar potency, inhibits the most closely related phosphatase (PTPRS) with 30-fold lower potency,and fails to display significant potency at sites assessed in EUROFINS assays. Neither heterozygous PTPRD knockout, acute 7-BIA administration nor two week repeated administration to mice provides any evidence for systemic or behavioral toxicities. Pretreatments with 7-BIA reduce cocaine self administration and conditioned place preference. To identify more potent and selective PTPRD phosphatase inhibitors, we have thus modeled the D1 phosphatase domains from PTPRD and related phosphatases and docked 7-BIA and other

candidate ligands to this site *in silico.* The central cysteine whose interactions with phosphotyrosine are key to the PTPRD's phosphatase activities are surrounded by amino acids that are highly conserved; drugs acting solely at this site appear unlikely to provide specificity. These observations are consistent with the lack of specificity for the smallest PTPRD phosphatase inhibitor, vanadate, that acts at virtually all phosphatases. However, amino acids at > 15 angstrom distances from the key PTPRD catalytic cysteine are less conserved; pharmacophores that interacted here could provide specificity and affinity. These observations are consistent with PTPRD's selectivity for substrates with specific amino acids found up to 9 amino acids distant from the substrate phosphotyrosine. Optimal, specific PTPRD phosphatase inhibitors for addiction pharmacotherapies may thus have "message" and "address" pharmacophores. *(support NIDA U01DA047713 and BRINM).*

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Poster

239. Neural Mechanisms of Addiction: Amphetamines

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 239.01/V1

Topic: G.08. Drugs of Abuse and Addiction

Support: National Institute of Health Grant DA029189 (TLW)

Title: Amphetamine-induced rise in glutamate in anterior cingulate correlates with positive incentive emotion in healthy women

Authors: *M. A. GONSALVES[1], T. L. WHITE[2];
[1]Neurosci. Grad. Program, [2]Ctr. for Alcohol & Addiction Studies, Brown Univ., Providence, RI

Abstract: Background: Glutamatergic antagonists alter subjective emotion in clinical samples. Glutamatergic agonists' effects are less well understood, and may inform the neural mechanisms of subjective emotion.
Methods: *D*-amphetamine (AMP; 20 mg), methamphetamine (MA; Desoxyn®, 20 mg), and placebo (PBO) were administered to 26 healthy adults in a double-blinded, within-subjects crossover design. Neurometabolites were assessed in the dorsal anterior cingulate (dACC) using proton magnetic spectroscopy (^{1}H MRS) 140-150 m post-drug. Emotion was evaluated using Positive and Negative Activation Rating Scales, Positive and Negative Affect Schedule, Visual Analogue Scales, Addiction Research Center Inventory, and Profile of Mood States, producing thirty one (31) measures x 8 time points x 3 sessions (744 data points per participant). Time-series data were reduced to area under the curve (AUC) scores; delta values summarized AUC differences under drug and PBO; and a principal components analysis (promax rotation) identified orthogonal factors of emotion induced by the study drugs. Drug effects on emotion and

neurometabolites were evaluated using pearson correlations.
Results: Emotion: AMP induced four factors of emotion: Activated Positive Affect, Somatic Sensations, Activated Negative Affect, and Anxious Distress. MA induced three factors of emotion: Activated Positive Affect, Activated Negative Affect, and Somatic + Low Negative Affect. Metabolic correlates: Females: AMP-induced changes in dACC Glu predicted the AMP-induced rise in activated positive affect, $r = .609$, $p < .05$. AMP-induced aversive Somatic Sensations was negatively correlated with AMP-induced changes in tCr, $r = -.721$, $p < .01$. Drug-induced changes in Gln correlated with Anxious Distress (AMP $r = .613$, $p < .05$), and Negative Affect (MA $r = -.705$, $p < .05$). Males: AMP-induced changes in Gln correlated negatively with Activated Negative Affect, $r = -.624$, $p < .05$.
Conclusions: Our results indicate a specific relationship between drug-induced changes in dACC glutamate and positive emotion in healthy women. These findings indicate a greater sensitivity to the positive emotional effects of psychostimulant-induced dACC glutamate in women, and a role for dACC glutamate in positive emotion.

Disclosures: M.A. Gonsalves: None. **T.L. White:** None.

Poster

239. Neural Mechanisms of Addiction: Amphetamines

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 239.02/V2

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH R01 DA019921
NIH R25 DA033674

Title: Opposing effects of glucocorticoids in the ventral hippocampus in stimulating accumbal dopamine release within amphetamine withdrawn vs. drug naïve rats

Authors: *G. L. FORSTER[1], K. A. CLEMENT[2], M. A. WEBER[3], B. BRAY[2];
[1]Univ. of Otago, Dunedin, New Zealand; [2]Basic Biomed. Sci., Univ. of South Dakota, Vermillion, SD; [3]Univ. of Iowa, Iowa City, IA

Abstract: Amphetamine withdrawal is characterized by increased stress sensitivity and dysphoria, thought to contribute to the chronic relapsing nature of amphetamine dependence. The purpose of our study was to elucidate neural mechanisms that may underlie psychological symptoms of amphetamine withdrawal. The ventral hippocampus is important for stress coping and influences the activity of a variety of other limbic-associated brain regions such as the nucleus accumbens. Here we show that a stress-relevant concentration of the glucocorticoid corticosterone (0.24 ng) infused into the ventral hippocampus of male rats stimulates accumbal dopamine release, as measured by *in vivo* chronoamperometry. This effect was dependent upon

both glucocorticoid and mineralocorticoid receptors in the ventral hippocampus, as pre-treatment with either mifepristone (2.91 nM) or spironolactone (2.99 nM) blocked the effect of corticosterone on accumbal dopamine release. Furthermore, infusions of BSA-conjugated corticosterone failed to replicate the effect of corticosterone alone, suggesting that the ability of corticosterone in the ventral hippocampus to elicit accumbal dopamine release is dependent on cytosolic receptors. Previously we observed reduced glucocorticoid receptor expression in the rat ventral hippocampus during amphetamine withdrawal. In line with this, the current study found that corticosterone (0.24 ng) infused into the ventral hippocampus of rats undergoing amphetamine withdrawal resulted in decreased accumbal dopamine levels. Overall, the ability of stress hormone within the ventral hippocampus to stimulate accumbal dopamine release may underlie, in part, the motivational effects of acute stressors. Moreover, stress hormone inhibition of accumbal dopamine release during amphetamine withdrawal may contribute to dysphoric states and the cycle of addiction.

Disclosures: G.L. Forster: None. **K.A. Clement:** None. **M.A. Weber:** None. **B. Bray:** None.

Poster

239. Neural Mechanisms of Addiction: Amphetamines

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 239.03/V3

Topic: G.08. Drugs of Abuse and Addiction

Title: D1 and D3 dopamine receptors coactivation enhances camp accumulation in low responding amphetamine treated rats, opposite effect in high responding rats

Authors: *D. G. MELCHOR, A. AVALOS, F. PAZ, B. FLORAN;
Ctr. De Investigacion Y De Estudios Avanzados De, Mexico City, Mexico

Abstract: Background: Recent studies reported that priming of L-DOPA in hemiparkonian rats generates two populations of animals, one *moderately dyskinetic*, neurochemically characterized by potentiation of D1 receptor stimulated accumulation of cAMP when are co-activated with D3 receptors. The other population; *severely dyskinetic*, in which D3 receptor antagonizes the D1 receptor. Similarities in molecular mechanisms evoked by L-DOPA and amphetamine (AMPH) priming suggest that effects on behavior and neurochemical outcome could be predicted. **Objectives:** The objective of this work were to evaluate behavior induced by amphetamine priming in rats and to characterize the effect of D1-D3 co-activation on cAMP accumulation in striatum. **Materials and methods:** We generated amphetamine priming in male Wistar rats (weight: 200-220 grs) administering 1mg/kg intraperitoneally each 24 hours for 5 days. Motor activity was determined using automated locomotion boxes. To evaluate effects of D1-D3 co-activation on cAMP accumulation, we used striatum slices treated with forskolin to stimulate cAMP as a control for AC activity. D1 and D3 receptors were activated by selective agonist. The

locomotor activity and cAMP accumulation results were analyzed by One-way ANOVA with proper post hoc test. **Results:** From 30 amphetamine-treated rats it was generated two populations: one with high activity (mean ambulatory distance by one our session: 22,867 cm; n = 17) and another with low activity (mean ambulatory distance: 12,050 cm; n= 13). Low activity group showed a significant increase in cAMP accumulation (43.8%; $p<0.001$) when co-activate D1-D3 receptors, compared with high activity in which one antagonistic interaction was obtained. **Conclusion:** These results demonstrate that amphetamine and L-DOPA priming present similar behavioral and neurochemical outcomes respect motor activity and D1 and D3 receptors interaction due probably to similar molecular mechanisms.

Disclosures: D.G. Melchor: None. **A. Avalos:** None. **F. Paz:** None. **B. Floran:** None.

Poster

239. Neural Mechanisms of Addiction: Amphetamines

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 239.04/V4

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA039145
NIH Grant DA031883

Title: Methylenedioxypyrovalerone (MDPV) increases plasmalemmal and vesicular dopamine transport

Authors: *C. P. MAGEE, C. L. GERMAN, Y. H. SIRIPATHANE, A. L. MARTIN, D. J. ANDERSON, G. R. HANSON, D. G. WILKINS, A. E. FLECKENSTEIN;
Univ. of Utah, Salt Lake City, UT

Abstract: Methylenedioxypyrovalerone (MDPV) is a synthetic cathinone that is abused by humans. Dysregulation of dopaminergic (DAergic) transporters such as the striatal dopamine transporter (DAT) and the vesicular monoamine transporter-2 (VMAT2) is key to psychostimulant self-administration and the development of psychostimulant-induced persistent DAergic deficits. Accordingly, the effects of MDPV on DAT and VMAT2 were investigated. *In vitro,* MDPV functions as a potent reuptake inhibitor at DAT (IC_{50} = 6.9 nM) whereas concentrations of 10 μM were without direct effect on VMAT2 activity. However, results reveal that *in vivo* MDPV administration (2.5 - 5 mg/kg, s.c.) rapidly (within 1 h) and reversibly *increases* both DAT and VMAT2 function. The MDPV-induced increase in DAT activity is noteworthy as plasma MDPV concentrations consequent to these doses far exceeded concentrations that directly decrease DAT activity *in vitro*. MDPV was detectable in plasma at 1, 3, and 6 h, but not 18 h after a single MDPV administration (2.5 mg/kg, s.c.). Additionally, MDPV was readily self-administered by rats (FR1, 0.0175 mg/infusion, 90-min sessions) and

detectable in plasma 1 h after the final self-administration session. Results revealed little evidence for a role for dopamine receptors in the MDPV-induced increases in DAT or VMAT2 function, as neither pretreatment with dopamine 1-like nor dopamine 2-like receptor antagonists attenuated the MDPV-induced increases in DAT or VMAT2 function. Further, despite a report that a non-selective nicotinic acetylcholine receptor (nAChR) antagonist attenuates MDPV self-administration, pretreatment with a nAChR antagonist did not attenuate the MDPV-induced increase in DAT function. Together with previous reports, these data suggest that the effects of MDPV at striatal DAergic transporters both resemble and are dissimilar to effects of other dopamine reuptake inhibitors such as methylphenidate and cocaine.

Disclosures: **C.P. Magee:** None. **C.L. German:** None. **Y.H. Siripathane:** None. **A.L. Martin:** None. **D.J. Anderson:** None. **G.R. Hanson:** None. **D.G. Wilkins:** None. **A.E. Fleckenstein:** None.

Poster

239. Neural Mechanisms of Addiction: Amphetamines

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 239.05/V5

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R33DA041876

Title: Cell-specific spinophilin function following psychostimulant-induced behavioral sensitization regimens

Authors: *D. S. WATKINS[1], A. J. BAUCUM II[2];
[1]Med. Neurosci., Indiana Univ. Sch. of Med., Indianapolis, IN; [2]Biol., Indiana University-Purdue Univ. Indianapolis, Indianapolis, IN

Abstract: Proper synaptic transmission is critical for maintaining neuronal communication. There is increasing evidence that in many striatal pathological disease-states such as Obsessive Compulsive Disorder (OCD), drug addiction and abuse, and others, synaptic transmission is perturbed. Furthermore, medium spiny neurons (MSNs) in many striatal-dependent disease-states exhibit differential perturbations in downstream signaling. Signal transduction pathways that are localized to the post-synaptic density (PSD) of the MSN regulate protein phosphorylation in a tightly controlled manner. Alterations in the control of this phosphorylation in striatal MSNs are observed in myriad striatal pathological disease-states. While serine/threonine kinases obtain substrate specificity, in part, by phosphorylating specific consensus sites, serine/threonine phosphatases such as protein phosphatase 1 (PP1), are much more promiscuous. To obtain substrate selectivity PP1 associates with targeting proteins. The major targeting protein in the PSD of dendritic spines is spinophilin. Spinophilin binds PP1 and

F-actin as well as multiple other synaptic proteins. Our lab has found that dopamine depletion, an animal model of PD, modulates spinophilin protein-protein interactions in the striatum. However, spinophilin function under hyperdopaminergic signaling such as those observed following psychostimulant-induced behavioral sensitization is less well characterized. To more specifically elucidate spinophilin function we have generated multiple transgenic animals that allow for cell- and age-specific loss of spinophilin as well as cell-specific interrogation of spinophilin protein interactions. Here, we begin to report the functional role of spinophilin in mediating behavioral changes associated with amphetamine-induced locomotor sensitization and delineate changes in spinophilin interactions that may mediate behavioral changes associated with amphetamine sensitization. Our data suggest that the spinophilin protein interactome is upregulated in MSNs following psychostimulant administration and that upregulation may be preferentially pronounced in different sexes. Furthermore, loss of spinophilin abrogates amphetamine-induced sensitization and plays a critical role in striatal motor learning. Together, our data suggests that spinophilin's protein-protein interactions in the striatum are obligate for normal striatal function. The implications for regulating spinophilin interactions in cell-specific MSNs as well as loss of spinophilin in regulating amphetamine-induced behavioral sensitization will be discussed.

Disclosures: D.S. Watkins: None. **A.J. Baucum II:** None.

Poster

239. Neural Mechanisms of Addiction: Amphetamines

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 239.06/V6

Topic: G.08. Drugs of Abuse and Addiction

Title: Chronic methamphetamine alters activation and expression patterns of CRF and CRFR1 neurons in stress-associated brain regions

Authors: *J. S. JACOBSKIND, Z. J. ROSINGER, R. M. DE GUZMAN, K. L. STURM, M. A. SARTORI, D. G. ZULOAGA;
State Univ. of New York at Albany, Albany, NY

Abstract: Corticotropin-releasing factor (CRF) binding to the CRF receptor-1 (CRFR1) subtype plays a key role in drug-related behaviors. Specifically, CRF signaling through CRFR1 increases drug seeking during abstinence and promotes stress-induced relapse to drug use. Previously, we found that MA challenge in mice with prior chronic exposure resulted in reduced activation of cells in paraventricular hypothalamus (PVN), extended amygdala, and other limbic regions. To explore the particular phenotypes of cells undergoing changes in activation, we i.p. injected male and female CRFR1-GFP and CRF-Cre/Ai9 reporter mice with MA (5 mg/kg) or saline for 9 consecutive days. The following day, all mice were given a challenge i.p. injection of MA

(5mg/kg) and sacrificed 120 minutes later. Neural activation patterns within CRF and CRFR1 cells were assessed by immunohistochemistry for c-Fos. CRFR1-GFP mice exposed to repeated MA displayed reduced activation of CRFR1 cells in the PVN. Further, males had a greater number of CRFR1-GFP cells in the PVN than females, regardless of treatment. CRFR1-GFP cell number was increased by chronic MA in the central amygdala (CeA) and ventral basolateral amygdala (vBLA), as was activation of CRFR1 cells in the vBLA. CRF-Cre/Ai9 mice exposed to repeated MA showed reduced activation of CRF cells in the PVN, and activation of PVN CRF neurons overall was greater in males than females. Regardless of treatment, few CRF neurons in the CeA and dorsolateral bed nucleus of the stria terminalis co-expressed c-Fos. Overall, these findings reveal a disruption to the CRF/CRFR1 system by repeated MA exposure. These changes may underlie behavioral modifications associated with MA withdrawal and relapse.

Disclosures: J.S. Jacobskind: None. **Z.J. Rosinger:** None. **R.M. De Guzman:** None. **K.L. Sturm:** None. **M.A. Sartori:** None. **D.G. Zuloaga:** None.

Poster

239. Neural Mechanisms of Addiction: Amphetamines

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 239.07/V7

Topic: G.08. Drugs of Abuse and Addiction

Support: The Helen M.C. Stern and J. Edward Stern Endowed Professorship in Psychology

Title: Behavioral evidence for the role of vesicular monoamine transporter in stimulant sensitization: The intracellular redistribution hypothesis

Authors: *A. VASQUEZ[1], V. GARCIA[1], S. A. COLLINS[1], P. PARADA[1], A. CRUZ[2], E. CASTAÑEDA[1];
[1]Psychology, Univ. of Texas At El Paso, El Paso, TX; [2]Psychological and Brain Sci., Texas A&M Univ., College Station, TX

Abstract: Behavioral sensitization is reflected as an increased response to the same dose of stimulant drug, such as amphetamine (AMPH). This phenomenon may drive the relentless craving and drug-seeking behavior in substance use disorders. Furthermore, behavioral sensitization to AMPH is mediated by a sensitization of dopamine (DA) overflow via an exchange diffusion mechanism at the Dopamine Transporter and subsequently at the Vesicular Monoamine Transporter (VMAT). However, the exact presynaptic mechanism responsible for neurochemical sensitization remains unknown. The research objective of this study was to examine whether VMAT might redistribute intracellular DA between the cytoplasmic compartment and vesicular stores. It was hypothesized that VMAT mediates DA availability between these two pools depending on the demand for overflow driven by the pharmacological

action of stimulant drugs versus the demand for exocytosis such as in response to drug-conditioned environmental cues. To test this, AMPH-evoked rotational behavior in a hemi-parkinsonian rat model was evaluated in rats pretreated with the VMAT blocker Tetrabenazine (TBZ). Thirty rats were randomly assigned to six groups: 1) Saline (SAL), 2) Low TBZ (0.5 mg/kg), 3) High TBZ (1.75 mg/kg), 4) AMPH (1.5 mg/kg), 5) L-TBZ + AMPH, and 6) H-TBZ + AMPH. For seven days, rats received an injection of SAL or TBZ followed by an injection of AMPH or SAL. Rotational behavior was immediately recorded by rotometers for 2 hr. A week after the sensitization phase, all rats received an AMPH challenge without TBZ. During the sensitizing phase, control groups receiving no AMPH showed minimal turning behavior and no extraneous effects of TBZ. In contrast, the AMPH group displayed a robust increase in turning behavior (ie, sensitization) on Day 7 compared to Day 1. Sensitization was dose-dependently blocked in the presence of TBZ. On the challenge day, all rats showed AMPH activation. The AMPH group displayed behavioral sensitization compared to the SAL group, which was dose-dependently blocked by a history of TBZ. In conclusion, VMAT may play a role in sensitization by redistributing intracellular pools of DA based on demand for overflow. Future studies will evaluate DA overflow evoked by AMPH and depolarizing stimulation.

Disclosures: **A. Vasquez:** None. **V. Garcia:** None. **S.A. Collins:** None. **P. Parada:** None. **A. Cruz:** None. **E. Castañeda:** None.

Poster

239. Neural Mechanisms of Addiction: Amphetamines

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 239.08/V8

Topic: G.08. Drugs of Abuse and Addiction

Support: UF McKnight Brain Institute Pilot Grant
NIH Grant T32DC015994

Title: Neuronal cell type-specific effects of primary cilia loss on stimulant-induced behaviors in mice

Authors: ***J. C. MCINTYRE**[1], C. A. RAMOS[1], J. B. ROBERTS[1], K. R. JASSO[1], A. K. FIREK[1], B. SETLOW[2];
[1]Neurosci., [2]Dept. of Psychiatry, Univ. of Florida, Gainesville, FL

Abstract: The neural mechanisms that regulate substance use disorders are complex, and impacted by a number of neuromodulatory peptides. Within the past ten years it has been discovered that several of the receptors for these neuromodulators are enriched in the primary cilia of neurons. Primary cilia are microtubule-based organelles that project from the surface of nearly all cells, including neurons. Despite what we know about cilia, our understanding of how

cilia regulate neuronal function and behavior is limited. One potential function for neuronal cilia is to provide a signaling platform for these neuromodulatory receptors. The primary goal of this study was to investigate how stimulants interact with cilia, and how cilia contribute to physiological responses to these substances. To first test this, we treated male C57bl6/j mice with 30mg/kg cocaine for 5 consecutive days. Twenty-four hours following the last injection, animals were euthanized and brains collected. Using immunofluorescent staining for the cilia-specific marker AC3, we found significantly shortened cilia in cocaine-treated mice (n=3) compared to saline-treated controls (n=3) in the ventral tegmental area (5.87μm vs 6.76μm, $p < 0.05$ $t=2.224$, $df=157$), nucleus accumbens (5.42μm vs 6.41μm, $p < 0.002$, $t=6.251$, $df=591$), and hippocampus (5.01μm vs 5.51μm, $p <0.01$, $t=2.716$ $df=334$). These data show that psychostimulants can modulate cilia morphology. To test the contributions of distinct cilia populations on stimulant-induced behaviors, we selectively ablated cilia from dopaminergic or GABAergic neurons in mice. Both female and male mice lacking cilia on dopaminergic neurons showed a significantly reduced locomotor response to acute administration of 3 mg/kg amphetamine compared to wildtype mice (females: $F(23, 264)=4.812$ $p<0.001$, males: $F(23, 624)=2.803$ $p<0.0001$). In contrast, female mice lacking cilia on GABAergic neurons showed similar responses to amphetamine as wildtypes ($F(23, 504)=0.4432$ $p=0.9893$), whereas male GABAergic cilia knockout mice showed significantly reduced locomotor activity ($F(23, 480)=2.467$ $p<0.0005$). Male GABAergic cilia knockout mice also showed enhanced sensitization to 1 mg/kg amphetamine over 5 days compared to wildtype mice ($F(1, 64) =14.45$ $p<0.0005$), an effect that was not observed in female knockout mice. In all cases cilia knockout mice showed no differences in baseline locomotor activity. These data indicate that cilia play critical roles in both acute responses to psychostimulants and psychostimulant-induced plasticity. Future work will focus on the signaling role of cilia in mediating neuropeptide modulation of responses to drugs of abuse.

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Poster

239. Neural Mechanisms of Addiction: Amphetamines

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 239.09/V9

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Fear 55490501

Title: Sex-specific differences in the role of central amygdala neuronal subtypes in drug-related behaviors

Authors: *A. BOUHUIS[1,2], B. LI[1];
[1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [2]Swammerdam Inst. for Life Sci., Univ. of Amsterdam, Amsterdam, Netherlands

Abstract: Methamphetamine is a psychostimulant drug that produces euphoria and induces behavioral activation through an increase in locomotion. Methamphetamine used to be prescribed as treatment for certain conditions, including attention deficit disorder, narcolepsy and obesity, but is increasingly being used recreationally. Due to its highly addictive nature, recreational methamphetamine use can quickly escalate to chronic methamphetamine abuse. Despite years of research on the mechanisms underlying methamphetamine addiction, there is no pharmacological treatment available and patients have to resort to solely behavioral therapies. To be able to identify appropriate pharmacological therapy targets, it is of importance to understand the acute and chronic effects methamphetamine has on the brain and how these effects can be translated to drug-related behaviors. Several studies have demonstrated a role for the central amygdala in different stages of methamphetamine use. However, no neuronal types have been identified within the central amygdala concerning methamphetamine abuse.
Using cell-type specific inhibitions in methamphetamine-induced conditioned place preference and on the elevated plus maze, we examine the role of two major cell populations in the central amygdala, Somatostatin (SST) and Prkc-delta (Prkcd), in the rewarding and anxiogenic properties of methamphetamine. In addition, we examine methamphetamine-induced synaptic changes in central amygdala slices by doing electrophysiological recordings. Our data suggests that central amygdala SST neurons are involved in the regulation of rewarding properties of methamphetamine, primarily in females. Furthermore, our electrophysiological data demonstrates a lasting (24 hour) effect from methamphetamine on SST neuronal synaptic activity, with contrasting effects across sexes. Prkcd neurons seem to be mediating the anxiogenic properties of methamphetamine in both males and females. These results show distinct roles for SST and Prkcd central amygdala neurons in methamphetamine-related behaviors, and they implicate gender in central amygdala SST neuronal regulation of the rewarding properties of methamphetamine.

Disclosures: **A. Bouhuis:** None. **B. Li:** None.

Poster

239. Neural Mechanisms of Addiction: Amphetamines

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 239.10/V10

Topic: G.08. Drugs of Abuse and Addiction

Support: University of Costa Rica/INISA Grant 742-B3-220
University of Costa Rica/IIP Grant 723-B7-610

Title: Changes in gene expression related to structural plasticity during the development and incubation of behavioral sensitization to amphetamine in rats

Authors: *V. ROJAS-GARBANZO[1], J. C. BRENES[2], A. SEQUEIRA-CORDERO[3];
[1]Neurosci. Res. Ctr., [2]Inst. for Psychological Res., [3]Inst. for Hlth. Res., Univ. of Costa Rica, San José, Costa Rica

Abstract: The transition to drug dependence is a multistep process between sporadic recreational drug use and the loss of control. Irresistible and compulsive drug intake and recurrent relapsing can be due to over-consolidation of memory mechanisms hijacked by continued drug abuse. Little is known, however, about the putative molecular targets underlying the structural brain changes related to psychostimulants dependence. D-amphetamine (AMP) is the prototypic psychostimulant that also constitutes a goal standard drug in psychopharmacology for modelling different psychiatric conditions. Our aim, therefore, was to study the behavioral response and the expression of genes related to neuronal plasticity (CRF, BDNF, TrkB, CREB, p250GAP, Cofilin 1, and Arp 2/3) in nucleus accumbens (NAc), dorsal striatum (DS), and hippocampus (HPC) of Wistar rats following sub-chronic exposure to AMP (seven 2.5 mg/Kg i.p injections in 12 days) or saline (SAL) (n=20 each). Thirty days after the last administration, SAL and AMP groups were divided into four groups (n=10 each) to get the following conditions: SAL-SAL, SAL-AMP, AMP-SAL, and AMP-AMP. Open-field behavior was monitored on each injection day. Each session consisted of a 15-minutes pre-test followed immediately by the injection and a 45-minutes post-test assessment. Forty-five minutes after testing, brain samples were obtained. Evidence will be presented regarding the development and incubation of behavioral sensitization in response to the context (15-minutes pre-test) or the drug (45-minutes post-test) and the likely associations between behavioral responses and gene expression in the brain regions analyzed.

Disclosures: V. Rojas-Garbanzo: None. **J.C. Brenes:** None. **A. Sequeira-Cordero:** None.

Poster

239. Neural Mechanisms of Addiction: Amphetamines

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 239.11/V11

Topic: G.08. Drugs of Abuse and Addiction

Title: The role of rearing diet on drug seeking behavior and DeltaFosB accumulation following amphetamine exposure

Authors: J. SAKACH, K. YANG, S. OO, ***K. PONDER**, A. M. ANCH;
St. Louis Univ., Saint Louis, MO

Abstract: High sugar diets and amphetamine use have both been evidenced to activate neural circuitry recognized in addiction while also showing outward drug seeking behavior. Previous research has demonstrated that amphetamine and excessive consumption of sucrose can induce accumulation of ΔFosB, a neurological marker for addiction. Conditioned place preference (CPP) has also shown associated reward-seeking behavior in response to amphetamine and high-sucrose diets. The present study aims to determine whether sucrose consumption during rearing contributes to adulthood amphetamine-seeking behavior as determined by CPP performance. Twenty male Sprague-Dawley rats were assigned to either a high-sugar diet or a normal chow diet following weaning. Once mature, rats underwent conditioned place preference trials, receiving injections of amphetamine and saline. Pilot data revealed no significant main effect of diet on amphetamine preference $F(1, 18)= 1.713$, $p=.210$, $\eta 2=.102$. There were no significant differences in total movement between diet conditions in acclimation, $t(17)= -.713$, $p=.486$, and in free association, $t(17)= -1.942$, $p=.063$. Histological analyses will be conducted to determine the differences in dietary groups on biomarkers of addiction.

Disclosures: J. Sakach: None. **K. Yang:** None. **S. Oo:** None. **K. Ponder:** None. **A.M. Anch:** None.

Poster

239. Neural Mechanisms of Addiction: Amphetamines

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Program #/Poster #: 239.12/V12

Topic: G.08. Drugs of Abuse and Addiction

Support: Department of Physiology and Pharmacology of Sapienza University
Development Fund 000126-2017
Sapienza University Fondi di Ateneo RM11715C457665A
NIDA Drug Supply Program, NIH

Title: Role of nucleus accumbens core but not shell in incubation of methamphetamine craving after voluntary abstinence

Authors: *L. ROSSI[1,2], I. REVERTE[1,2], J. MODONI[3], D. RAGOZZINO[1], A. BADIANI[1,4], M. VENNIRO[5], D. CAPRIOLI[1,2];
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Abstract: We recently introduced an animal model to study incubation of drug craving after prolonged voluntary abstinence, mimicking the human condition of relapse after successful

contingency management treatment. Here we studied the role of the nucleus accumbens (NAc) in this model. We trained rats to self-administer a palatable solution (sucrose+maltodextrin 1%, 6 h/d, 6 d) and methamphetamine (6 h/d, 12 d). We then evaluated relapse to methamphetamine seeking after 1 and 15 days of voluntary abstinence, achieved via a discrete choice procedure between the palatable solution and methamphetamine (14 d). We used RNAscope in-situ hybridization to quantify the colabeling of the neuronal activity marker Fos, and dopamine Drd1- and Drd2 expressing medium spiny neurons (MSNs) in NAc core and shell during the incubation tests. Next, we determined the effect of pharmacological inactivation of NAc core and shell by either $GABA_A$ and $GABA_B$ agonists (muscimol+baclofen, 50+50 ng/side) or selective Drd1 and Drd2 antagonists (SCH39166 1.0 μg/side, raclopride 1.0 μg/side) during the relapse tests. Incubated methamphetamine seeking after voluntary abstinence was associated with a selective increase of Fos expression in the NAc core, but not shell, and Fos was co-labeled with both Drd1- and Drd2-MSNs. NAc core, but not shell, injections of muscimol+baclofen, SCH39166, and raclopride agonists reduced methamphetamine seeking after 15 days of abstinence. Together, our results suggest that dopamine transmission through Drd1 and Drd2 in NAc core is critical to the incubation of methamphetamine craving after voluntary abstinence.

Disclosures: **L. Rossi:** None. **I. Reverte:** None. **J. Modoni:** None. **D. Ragozzino:** None. **A. Badiani:** None. **M. Venniro:** None. **D. Caprioli:** None.

Poster

239. Neural Mechanisms of Addiction: Amphetamines

Location: Hall A

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Topic: G.08. Drugs of Abuse and Addiction

Support: Development Fund 000126-2017, Department of Physiology and Pharmacology, Sapienza University of Rome
Fondi Ateneo RM11715C457665A, Sapienza University of Rome
NIDA Drug Supply Program, NIH

Title: Effect of social choice-induced voluntary abstinence on incubation of methamphetamine craving and glutamate receptor expression in nucleus accumbens core

Authors: *I. REVERTE[1,4], L. M. ROSSI[1,5], J. MODONI[5], L. LO IACONO[2,5], M. FULLONE[3], R. MIELE[3], M. VENNIRO[6], D. CAPRIOLI[1];
[1]Physiol. and Pharmacol., [2]Dept. of Psychology, [3]Dept. of Biochem. Sci., Sapienza Univ. of Rome, Rome, Italy; [4]IRCSS Santa Lucia Fndn., Rome, Italy; [5]IRCCS Santa Lucia Fndn., Rome, Italy; [6]Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: *Rationale:* Cue-induced cocaine and methamphetamine (Meth) craving progressively intensifies during forced abstinence from drug self-administration (a phenomenon termed incubation of craving). Previous electrophysiological studies revealed that incubation of cocaine and Meth craving is associated with an accumulation of GluA2-lacking calcium-permeable AMPA receptors (CP-AMPARs). Biochemical studies on cocaine incubation suggest that the CP-AMPA are mainly homomeric GluA1 as reflected by an increased cell surface of the GluA1 subunit. Here we quantified AMPARs subunits (GluA1, GluA2 and GluA3) in Meth trained rats after forced or social choice-based voluntary abstinence, the latter a procedure that attenuates the emergence of incubation of Meth craving.
Methods: We trained rats to lever press for a social-peer (social self-administration; 2h/d, 5 d) and subsequently to intravenous self-administration of Meth (6 h/d, 12 d) or saline (control groups). We then evaluated relapse to Meth seeking after 1 and 15 days of forced or social voluntary abstinence, the latter achieved via a discrete choice procedure between the social peer and Meth (14 d). Immediately after the relapse tests on day 1 and 15, we dissected the NAc core for the quantification of AMPARs subunits. We used a BS3 crosslinking procedure, which enables the distinctive quantification of surface and intracellular proteins by Western Blotting.
Results: In agreement with our previous results Meth seeking was higher on day 15 than on day 1 (incubation of craving) after forced but not social choice-induced voluntary abstinence. Neither incubation after forced abstinence nor blockade of incubation by social choice-induced voluntary abstinence were associated with changes in levels of surface AMPARs subunits: GluA1, GluA2 and GluA3, which were not different from those of drug-naïve control rats on abstinence days 1 and 15.
Conclusions: Our preliminary biochemical results suggest that incubation of Meth craving after forced abstinence or the blockaded incubation of Meth craving after social choice-induced abstinence are not associated with evident changes in AMPARs expression in nucleus accumbens core.

Disclosures: I. Reverte: None. **L.M. Rossi:** None. **J. Modoni:** None. **L. Lo Iacono:** None. **M. Fullone:** None. **R. Miele:** None. **M. Venniro:** None. **D. Caprioli:** None.

Poster

239. Neural Mechanisms of Addiction: Amphetamines

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 239.14/V14

Topic: G.08. Drugs of Abuse and Addiction

Support: Regis University SRSG and FRSG support

Title: The effect of exercise on relapse to methamphetamine seeking

Authors: *H. R. JENKINS, A. S. PAINE, B. R. FINE, A. N. FRICKS-GLEASON;
Regis Univ., Denver, CO

Abstract: Methamphetamine (METH) is a widely abused neurotoxic stimulant. Abuse of METH costs the government billions of dollars each year, due to lost work place productivity and crime and wreaks havoc in the lives of users due to its side effects which can include: psychosis, violent behavior, physical deterioration, depression and cognitive impairment. In addition to its negative behavioral effects, METH is also a neurotoxin, which causes cell injury in specific dopaminergic brain regions such as the striatum. Even if users are able to seek and successfully complete treatment, there is a high risk of relapse: one study found that relapse rates for METH users were as high as 61% during the first year post-treatment and 25% during the 2-5 years post-treatment. Therefore, it is important to find an effective and easily implemented therapy for METH users that can protect against the high risk of relapse. While exercise is already well known for its physical and cognitive health benefits, previous work has also found that exercise can attenuate METH-induced neurotoxicity as well as METH-induced cognitive impairments. However, no work has yet been done to study the effects of exercise on METH relapse. Conditioned place preference (CPP) is a paradigm used to study relapse in an animal model by modeling the impact of drug-paired cues on drug seeking behavior. Studies have demonstrated the ability of exercise to decrease CPP for both cocaine and amphetamine, but no studies have investigated the effects of exercise on METH CPP. The present study examined the effects of voluntary exercise on extinction and (drug- or stress-primed) reinstatement of METH conditioned place preference in rats.

Disclosures: H.R. Jenkins: None. **A.S. Paine:** None. **B.R. Fine:** None. **A.N. Fricks-Gleason:** None.

Poster

239. Neural Mechanisms of Addiction: Amphetamines

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 239.15/V15

Topic: G.08. Drugs of Abuse and Addiction

Support: Regis University FRSG & SRSG Support

Title: The effects of exercise on methamphetamine-induced cognitive deficits

Authors: *A. S. PAINE, H. R. JENKINS, B. R. FINE, A. N. FRICKS-GLEASON;
Regis Univ., Denver, CO

Abstract: Methamphetamine (METH) abuse is problematic and prevalent with nearly 60 million people abusing METH worldwide. METH abuse leads to delusions, aggressive behavior, and

addiction in users, causing major social and economic problems. Additionally, METH induces neurotoxicity in the brain, specifically in the prefrontal cortex, basal ganglia and hippocampus, as well as leading to cognitive deficits similar to those seen in Parkinson's disease. There is currently no effective behavioral or pharmacological treatment for METH abuse. Exercise is well known for its physiological and cognitive enhancing properties, and it has been shown to be an effective therapy for neurodegenerative diseases such as Parkinson's. Due to the similarities between Parkinson's and METH abuse it is possible exercise will be an effective and low cost therapy for drug addiction as well. The goal of this study is to research exercise as an effective way to attenuate METH-induced cognitive deficits, which we will do by using a well validated rat model of METH-induced neurotoxicity and cognitive tasks such as object-in-place and an eight arm radial arm maze.

Disclosures: A.S. Paine: None. **H.R. Jenkins:** None. **B.R. Fine:** None. **A.N. Fricks-Gleason:** None.

Poster

239. Neural Mechanisms of Addiction: Amphetamines

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 239.16/V16

Topic: G.08. Drugs of Abuse and Addiction

Support: Regis University SRSG and FRSG support

Title: Elucidating the mechanisms of exercise-induced attenuation of methamphetamine neurotoxicity and cognitive deficits

Authors: *B. R. FINE, H. R. JENKINS, A. S. PAINE, A. N. FRICKS-GLEASON;
Dept. of Psychology & Neurosci., Regis Univ., Denver, CO

Abstract: Methamphetamine abuse is a significant public health concern, largely due to the neurotoxicity that results from use. Previous research from our lab has demonstrated that exercise can attenuate methamphetamine neurotoxicity. Furthermore, we've shown that exercise may increase levels of brain derived neurotrophic factor (BDNF) during attenuation of neurotoxicity (Murray et al., 2016). This suggests that the negative effects of METH can be "reversed" and that BDNF may play a role in that attenuation. Levels of BDNF, and other growth factors, have been shown to increase with exercise. Expression of these growth factors has also been positively correlated with improved cognitive function when paired with exercise after damage (Cotman and Engesser-Cesar, 2002; Cotman and Berchtold, 2002). Here, we aim to show correlations between exercise-induced attenuation of METH neurotoxicity, cognitive abilities, and the levels of different growth factors. This project will expand upon previous results from our lab and apply them to a low-cost way to combat the effects of the METH use

and the cognitive deficits these users have suffered. Because exercise is an easily attainable (for a general population) activity that can reverse METH-induced cognitive deficits, it is a promising therapeutic for the recovering addict population. For the pre-clinical research community, finding the underlying mechanisms behind why the exercise is working to "reverse" these negative consequences is something that would greatly add to the existing literature and would pave the way for future research into additional therapeutic aids for those who are trying to recover from an addiction to METH.

Disclosures: B.R. Fine: None. **H.R. Jenkins:** None. **A.S. Paine:** None. **A.N. Fricks-Gleason:** None.

Poster

239. Neural Mechanisms of Addiction: Amphetamines

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 239.17/V17

Topic: G.08. Drugs of Abuse and Addiction

Support: VA Merit grant BX003304

Title: *In vivo* reduction of striatal D1 receptors by RNA interference is associated with changes in methamphetamine self-administration and intracellular protein expression

Authors: *A. D. KREISLER[1], M. J. TERRANOVA[1], S. S. SOMKUWAR[2], D. C. PUROHIT[1], S. WANG[1], B. P. HEAD[1], C. D. MANDYAM[1];
[1]VA San Diego Healthcare Syst., San Diego, CA; [2]VA San Diego, San Diego, CA

Abstract: The dorsal striatum (DS) is important for the development of drug addiction; however, the role of dopamine D1 receptors (D1Rs) in regulating excessive methamphetamine intake remains elusive. Here we seek to determine if reducing D1R expression in the DS via RNA interference alters methamphetamine self-administration. A lentiviral vector-mediated approach was used to overexpress short hairpin RNA (shRNA) against D1Rs (LV-shD1R) or scrambled control virus in the DS. LV-shD1R treatment in male rats increased responding for methamphetamine (0.05 mg/kg, i.v.) under a fixed-ratio schedule in an extended access paradigm, compared to scrambled controls. LV-shD1R also produced a vertical shift in self-administration during a dose-response paradigm and reduced responding for methamphetamine in a progressive-ratio schedule of reinforcement. LV-shD1R did not alter responding for sucrose (oral) under a fixed-ratio schedule. Western blotting analysis confirmed reduced D1R expression in LV-shD1R rats; expression of synaptic proteins including PSD-95 and MAPK-1 was reduced in the DS of methamphetamine-exposed, but not sucrose-exposed rats. Our studies indicate that reduced D1R expression-mediated changes in methamphetamine self-administration is associated with cellular adaptations that support dysfunctional dopamine signaling in the DS.

Disclosures: **A.D. Kreisler:** None. **M.J. Terranova:** None. **S.S. Somkuwar:** None. **D.C. Purohit:** None. **S. Wang:** None. **B.P. Head:** None. **C.D. Mandyam:** None.

Poster

239. Neural Mechanisms of Addiction: Amphetamines

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 239.18/V18

Topic: G.08. Drugs of Abuse and Addiction

Support: National Institute Drug Abuse DA034116

Title: Methamphetamine learning induces persistent nonmuscle myosin II-dependent spine motility in the basolater amygdala

Authors: *E. J. YOUNG[1], G. RUMBAUGH[2], C. A. MILLER[1];
[1]Mol. Med. and Neurosci., [2]Neurosci., Scripps Res. Inst., Jupiter, FL

Abstract: Nonmuscle myosin II inhibition (NMIIi) in the basolateral amygdala (BLA) selectively disrupts memories associated with methamphetamine (METH) days after learning, without retrieval. However, the molecular mechanisms underlying this selective vulnerability remain poorly understood. A known function of NMII is to transiently activate dendritic spine actin dynamics with learning. Therefore, we hypothesized that METH-associated learning perpetuates NMII-driven actin dynamics in dendritic spines, leading to an extended window of vulnerability for memory disruption. Two-photon imaging of actin-mediated spine motility in neurons from memory-related structures, BLA and CA1, revealed a persistent increase in spine motility after METH-associated learning that was restricted to BLA neurons. METH-induced changes to BLA spine dynamics were reversed by a single systemic injection of an NMII inhibitor. Thus, a perpetual form of NMII-driven spine actin dynamics in BLA neurons may contribute to the unique susceptibility of METH-associated memories.

Disclosures: **E.J. Young:** None. **G. Rumbaugh:** None. **C.A. Miller:** None.

Poster

240. Opioids: Mechanisms of Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 240.01/V19

Topic: G.08. Drugs of Abuse and Addiction

Support: This research was supported by the P50 grant DA027840 from NIDA.

Title: Acute and chronic amitifadine, a triple monoaminergic re-uptake inhibitor reduces self-administration of both nicotine and the opiate remifentanil

Authors: *A. H. REZVANI, C. WELLS, G. BLAIR, A. VIERLING, J. ROSE, E. D. LEVIN; Duke Univ., Durham, NC

Abstract: A variety of neural systems are involved in the basis of drug addiction. Although drugs of abuse have a variety of different modes of action, there are some commonalities with their neural bases. We have found several different types of drug treatments that successfully reduce nicotine self-administration in a rat model. The current studies are the first in a series to determine if drug treatments that have been found to significantly reduce nicotine self-administration would also reduce opiate self-administration. Acute and chronic treatment of young adult female rats with amitifadine, which inhibits the reuptake of dopamine, norepinephrine and serotonin, was shown in our studies to significantly reduce self-administration of the opiate remifentanil. Acutely, amitifadine at doses of 5, 10 and 20 mg/kg significantly reduced remifentanil self-administration. In the chronic study, repeated treatment with 10 mg/kg of amitifadine continued reducing remifentanil self-administration. The 10 mg/kg amitifadine dose did not significantly affect food motivated responding and did not attenuate remifentanil-induced analgesia as measured on the hot plate test. Rather, amitifadine itself had a moderate but lasting analgesic effect. These studies show the promise of amitifadine as a treatment for countering opiate self-administration. Further studies are needed to determine the possible efficacy of amitifadine for combating opiate addiction in people.

Disclosures: A.H. Rezvani: None. **C. Wells:** None. **G. Blair:** None. **A. Vierling:** None. **J. Rose:** None. **E.D. Levin:** None.

Poster

240. Opioids: Mechanisms of Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 240.02/V20

Topic: G.08. Drugs of Abuse and Addiction

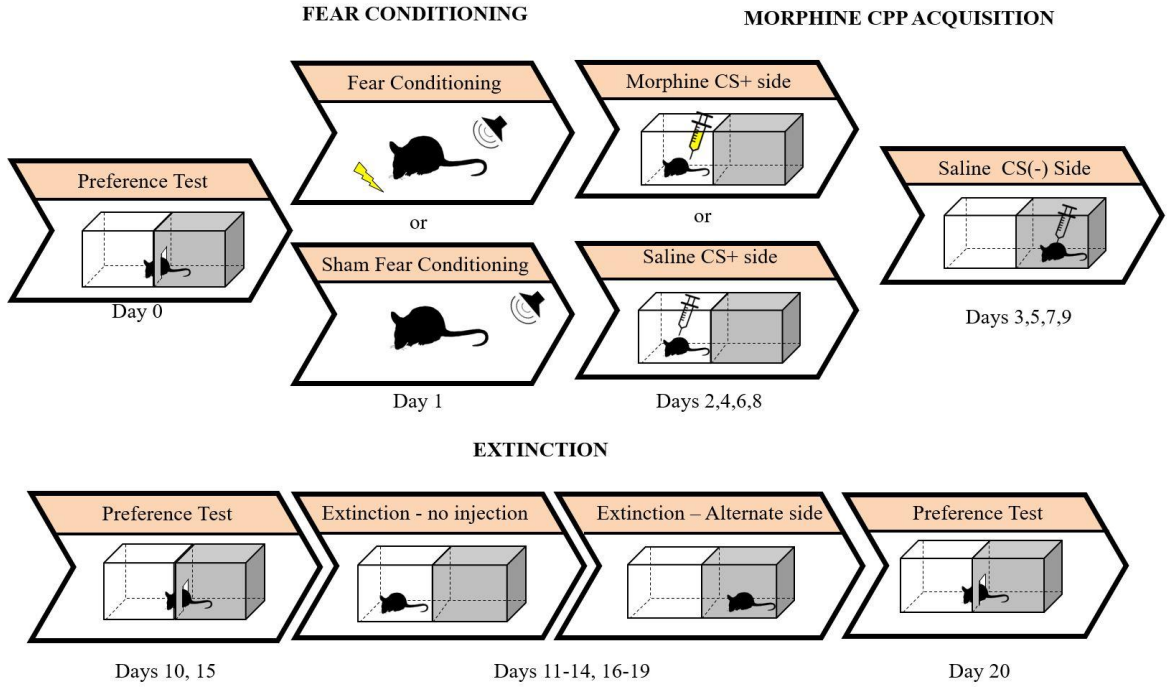
Support: VA Merit Review Award I01RX001144
VA Interprofessional Polytrauma and Traumatic Brain Injury Rehabilitation Research Fellowship
NIH/NIDA Grant T32DA041898

Title: Impact of fear conditioning on extinction of morphine conditioned place preference in mouse

Authors: *N. L. JOHNSON[1], J. A. BABB[2], G. B. KAPLAN[3];
[1]Boston Univ. Sch. of Publ. Hlth., Boston, MA; [2]Res. Mailstop 151, VA Boston Healthcare System/Harvard Med. Sch., West Roxbury, MA; [3]Psychiatry and Pharmacol., VA Boston Healthcare System/Boston Univ. Sch. Med., Brockton, MA

Abstract: Close to 3 million Americans meet the criteria for having an opioid use disorder (OUD), which remains an ongoing epidemic. Around 40% of the population with an OUD has a co-occurring psychiatric disorder, and among these, posttraumatic stress disorder (PTSD) is the most prevalent. In this project, we utilized two well-known animal models, conditioned place preference (CPP), and fear conditioning (FC) to study the impact of prior aversive learning on associative learning to morphine in adult male C57BJ/6 mice. All mice that were exposed to footshock acquired robust conditioned fear responses after six pairings of a tone cue associated with footshock, compared to control groups which were only exposed to the tone cue. Subsequently, all mice were exposed to morphine associated CPP and were compared to saline-treated controls. Fear conditioned mice acquired morphine-induced CPP to a similar degree as non-fear conditioned control mice. Preliminary data suggests that fear conditioned mice extinguished morphine CPP faster than non-fear-conditioned mice; extinction was accelerated by ~50% compared to mice who did not receive FC. These data suggest that prior aversive learning may attenuate learning of future appetitive associations. Ongoing experiments are investigating the underlying neural mechanisms of this behavioral effect. These findings can inform our understanding of the neurobiological mechanisms underlying PTSD-OUD comorbidity and improve treatment research into these disorders.

Schematic of co-morbid model of PTSD-OUD



Disclosures: **N.L. Johnson:** None. **J.A. Babb:** None. **G.B. Kaplan:** None.

Poster

240. Opioids: Mechanisms of Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 240.03/V21

Topic: G.08. Drugs of Abuse and Addiction

Title: Optogenetic stimulation of the NAc - VTA GABAergic pathway is rewarding and potentiates heroin-taking and seeking

Authors: *E. J. GALAJ, C. JORDAN, Z.-X. XI;
MTMD/ Addiction Biol. Unit, NIDA IRP, Baltimore, MD

Abstract: Despite extensive research in the past half a century, the neural mechanisms underlying opioid reward and addiction are still not fully understood. A commonly accepted view is that opioid reward is mediated by opioid-induced reduction in the extracellular GABA release in the ventral tegmental area (VTA) that subsequently disinhibits (aka activates) VTA dopamine (DA) neurons. However, the findings in literature are conflicting, and the role of DA in opioid reward is still debatable. Among GABAergic inputs, VTA DA neurons receive major inhibitory inputs from local interneurons and from the nucleus accumbens (NAc) medium-spiny (MSN) neurons. Using transgenic and optogenetic techniques, here we dissected the role of VTA DA neurons, GABA interneurons, and NAc-GABA inputs to the VTA in opioid reward. In consistent with previous reports, optogenetic stimulation of VTA DA neurons was found to be rewarding in mice, while activation of VTA GABAergic interneurons to be aversive, as assessed by optical intracranial self-stimulation (oICSS) and real-time place preference (oRTPP). As expected, optical inactivation of VTA DA neurons produced significant reductions in heroin self-administration and reinstatement of drug-seeking, providing supporting evidence for an important role of DA in opioid reward. Optical activation of VTA GABA interneurons did not produce significant reductions in heroin self-administration. Surprisingly, optical stimulation of NAc-GABA inputs to the VTA produced robust rewarding effects, as assessed by oICSS and oRTPP, and potentiated intravenous heroin self-administration and cue-induced reinstatement of heroin-seeking. These findings suggest that not all GABA inputs form direct inhibitory synapses with VTA DA neurons. Specifically, GABAergic afferents derived from the NAc may form synapses on other non-DA neurons (most likely on the VTA GABA neurons), producing rewarding effects. These unexpected findings inspire us to further study 1) which type of NAc neurons - D1-MSNs or D2-MSNs are part of the GABA input to the VTA that produces rewarding effects, as observed in the present study; 2) which type of VTA neurons (DA neurons or GABA interneurons) forms functional synapses with the NAc-VTA GABAergic afferents; and 3) which cell type-specific mechanisms (DA neurons and/or GABAergic interneurons or perhaps afferents) underlie opioid reward.

Disclosures: **E.J. Galaj:** None. **C. Jordan:** None. **Z. Xi:** None.

Poster

240. Opioids: Mechanisms of Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 240.04/V22

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH P01DA047233 (to EJN)
Natural Sciences and Engineering Research Council of Canada Postdoctoral Fellowship (to CJB)

Title: Transcriptional reprogramming of the brain reward system by heroin self-administration

Authors: *C. J. BROWNE, R. FUTAMURA, A. GODINO, F. MARTÍNEZ-RIVERA, A. MINIER-TORIBIO, A. RAMAKRISHNAN, A. TORRES-BERRÍO, E. M. PARISE, D. M. WALKER, L. SHEN, E. J. NESTLER;
Nash Family Dept. of Neuroscience, Friedman Brain Inst., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Opioid abuse exacts a devastating toll on individuals, their families, and the healthcare system as a whole. Treating opioid addiction is exceptionally difficult because, even after prolonged abstinence, re-exposure to the drug or to drug-associated cues can trigger relapse to compulsive drug-seeking. The persistence of opioid-induced behavioral abnormalities is mediated in part by changes to gene expression programs within interconnected regions of the brain involved in reward-processing. Although previous studies have identified several candidate genes regulated by opioids, no studies have comprehensively examined transcriptome-wide changes across the reward system following volitional opioid intake. Here, we combine heroin self-administration in mice, next-generation RNA sequencing (RNA-seq), and bioinformatic analyses to identify novel genes and gene networks throughout the reward system that are regulated by opioid abuse. This approach has been employed by our lab to identify key regulators of cocaine self-administration (Walker et al., 2018, *Biol Psych*). First, mice were trained to self-administer heroin (0.05 mg/kg/infusion) on a fixed-ratio 1 schedule of reinforcement in 15 daily 4h sessions. Mice were then euthanized either 24h after the last self-administration session or following a 30-day withdrawal period. In the 30-day group, mice received either a saline or heroin challenge (1 mg/kg, SC) and were placed back into self-administration chambers to measure drug-primed and/or context-induced reinstatement of heroin-seeking under extinction conditions for 2h, after which mice were immediately euthanized. Six brain regions involved in various aspects of reward-processing were collected and processed for RNA-seq: prefrontal cortex, nucleus accumbens, dorsal striatum, basolateral amygdala, ventral hippocampus, and ventral tegmental area. We are currently analyzing this

dataset to identify key driver genes and gene networks altered by opioid exposure, and correlating these transcriptional changes with behavioral measures that model aspects of opioid abuse. These studies will provide fundamental insights into opioid-induced transcriptional regulation, and can be contrasted with RNA-seq studies of cocaine to delineate drug-shared and drug-distinct molecular signatures of addiction.

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Poster

240. Opioids: Mechanisms of Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 240.05/V23

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant 2018 UG3 DA

Title: Single cell sequencing of the medial habenula in response to oxycodone self-administration

Authors: *C. FILLINGER, R. SEBRA, A. HAKE, K. G. BEAUMONT, M. WILLIAMS, P. J. KENNY;
Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: More than 197 million prescriptions for opioid painkillers were dispensed in the United States in 2017 alone. This unprecedented level of opioid consumption has led to an epidemic of opioid overdose and death. One of the most prescribed opioids worldwide is Oxycodone (Oxy), a strong semisynthetic painkiller derived from opium. Little is known about the actions of Oxy in the brain compared with other opioids and, more broadly, the precise brain circuitries that contribute to opioid addiction are poorly understood. The medial habenula (MHb) is strategically placed in the brain to serve as a major relay between limbic prefrontal structures and the neuromodulatory regions of the midbrain and brainstem, integrating cognitive, emotional and sensorial information. Moreover, the MHb contains the highest density of mu-opioid receptors in the brain and has been shown to play a critical role in regulating the motivational properties of other drugs of abuse such as nicotine. Based on these considerations, we hypothesize that the MHb plays an important role in the addiction-relevant actions of Oxy and other opioid drugs. As a first step to testing this hypothesis, we established an intravenous Oxy self-administration procedure for mice and used this procedure to characterize transcriptional plasticity in the MHb in response to Oxy intake using single cell sequencing. First, we assessed responding for Oxy in mice when escalating doses of the drug were available under a fixed-ratio

5 schedule of reinforcement. Specifically, mice had access to a dose of 0.1 mg/kg/infusion for 6 days. After stabilization of intake, mice then had access to a dose of 0.3 mg/kg/infusion for 6 days, followed by a dose of 0.6 mg/kg/infusion for 5 days. Control animals had access to food rewards only. On the last day, we withheld Oxy access and assessed seeking-like responses for the drug. Immediately after this last session, we collected habenula tissues from all mice and performed single-cell RNA sequencing to profile the effect of Oxy self-administration on the habenula transcriptome. This approach will bring new insight about potential brain alterations induced by a prolonged consumption of Oxy. Moreover, it may facilitate identification of novel targets for medications development in the MHb that will aid in the fight against the opioid addiction.

Disclosures: C. Fillinger: None. **R. Sebra:** None. **A. Hake:** None. **K.G. Beaumont:** None. **M. Williams:** None. **P.J. Kenny:** None.

Poster

240. Opioids: Mechanisms of Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 240.06/V24

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R01 DA035281

Title: Changes in intracranial self-stimulation following intermittent abstinence during oxycodone self-administration are associated with kappa opioid receptor signaling

Authors: *J. D. NGUYEN, Y. GRANT, M. A. TAFFE;
Univ. of California San Diego, La Jolla, CA

Abstract: Prescription opioid abuse is a significant global health problem characterized by persistent drug-seeking despite adverse consequences on health and well-being. The negative reinforcement hypothesis proposes that continued drug-taking is driven in part by the dysphoric or negative affective state experienced during daily cycles of drug withdrawal. Previous investigations indicate that elevations in intracranial self-stimulation (ICSS) reward thresholds may index the anhedonic symptoms associated with withdrawal from drugs of abuse, including illicit opioids. In this study, we used the ICSS procedure to determine if dysphoria is associated with the escalation of intravenous self-administration of oxycodone. Adult male Wistar rats were implanted with unilateral electrodes aimed at the medial forebrain bundle and trained in a discrete-trial current-threshold procedure. The rats were later implanted with intravenous catheters and trained to self-administer oxycodone (0.15 mg/kg/infusion) under short (1 h; ShA) or extended (11 h; LgA) access conditions. Rats were tested for ICSS thresholds ~1 h prior to oxycodone self-administration sessions. A separate group of rats were administered norBNI (30

mg/kg, i.p.) or saline vehicle prior to self-administration testing. Mean oxycodone infusions obtained under LgA conditions were significantly higher, compared with ShA conditions, and the 60 h weekend abstinence periods further increased drug intake in LgA rats. Pre-session brain reward thresholds increased across successive weekdays in LgA rats, however 1 h of oxycodone self-administration was sufficient to return reward thresholds to baseline. ICSS thresholds were also restored towards baseline across 60 h weekend abstinence, however there was no reward-facilitating effect of oxycodone self-administration after 60 h withdrawals. The increase in ICSS thresholds was attenuated by systemic administration of norBNI. We found that the duration of daily drug access and discontinuation each impacted the acquisition and maintenance of self-administration of oxycodone. Overall, these data suggest that escalation of self-administration of oxycodone is driven by a complex mixture of negative and positive reinforcement following intermittent abstinence and may be associated with kappa opioid receptor-mediated changes in sensitivity of brain reward status.

Disclosures: **J.D. Nguyen:** None. **Y. Grant:** None. **M.A. Taffe:** None.

Poster

240. Opioids: Mechanisms of Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 240.07/V25

Topic: G.08. Drugs of Abuse and Addiction

Title: The opioid agonist remifentanil does not induce subjective pleasure pre-surgery: An observational open-label study

Authors: *S. LEKNES[1], G. ERNST[1], R. MEIR[1], M. EIKEMO[2];
[1]Univ. Oslo, Oslo, Norway; [2]Univ. of Oslo, Oslo, Norway

Abstract: Opioid agonists are known for their analgesic and rewarding effects. Neurobiological models of addiction explain that initial drug liking and euphoria subsides over time with repeated opioid drug use, whereas drug craving increases. However, opioid drugs given to healthy, non-abusing people in laboratory settings often elicit modest or no drug-induced euphoria, with many participants reporting disliking the opioid drug effects. To understand the rewarding and addictive properties of opioid agonists, ecologically valid data from clinical opioid use is needed. Opioid drugs are also frequently used for stress relief, e.g. on the operating table prior to anesthesia. In an observational open-label study, we assessed current affective state and drug effects in day surgery patients treated at a non-university hospital in Norway. Remifentanil was administered at an effect-site concentration of 5ng/ml using a target-controlled infusion model (Minto) which is based on sex, age, weight and height. Patients rated their levels of feeling good and anxious on an 11-point numerical rating scale immediately before and 1-2 minutes after receiving remifentanil infusion. They also rated feeling high, liking the drug effects and their

level of drug-related discomfort.
Data from 123 patients (mean age 46, 75 women) undergoing minor orthopedic, abdominal, colorectal, gynaecological and otorhinolaryngological surgery was analysed using t-tests and mixed ANOVAs. Patients were relatively healthy and average ongoing pain before operation was only 1.2 on an 11-point scale. After remifentanil, patients reported feeling high (mean ± SD: 6.4 ± 2.1). Somewhat surprisingly, patients felt on average 0.5 points less good after remifentanil (pre: 6.9 ± 2.1; post: 6.4 ± 2.3, t (122) = 2.79, p=0.006, BF10 = 4.02). Also, pre-surgery remifentanil did not significantly reduce anxiety (pre: 3.2 ± 2.7; post: 3.0 ± 2.8, t (116) = 1.54, p=0.125, BF01 = 3.1). Neither ratings of feeling good or feeling anxious were significantly moderated by the presence of pain (44% reported any current pain) or opioid use status (30 % of patients reported being opioid naïve).
In sum, the fast-acting mu-opioid agonist remifentanil does not appear to cause euphoria or stress (anxiety) relief when administered immediately before surgery.

Disclosures: **S. Leknes:** None. **G. Ernst:** None. **R. Meir:** None. **M. Eikemo:** None.

Poster

240. Opioids: Mechanisms of Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 240.08/V26

Topic: G.08. Drugs of Abuse and Addiction

Title: One trial fentanyl-induced sensitization in adolescent and adult rats

Authors: O. OROZCO, H. DIAZ, D. L. SANCHEZ, *C. A. CRAWFORD;
California State Univ., San Bernardino, CA

Abstract: Opioid misuse has reached epidemic proportions in the United States, primarily as a result of the rise in availability of synthetic prescription opioids like fentanyl. Despite the growing problems of fentanyl abuse, few preclinical investigations have assessed the addictive properties of this compound. Thus, the goal of the current study was to assess the abuse liability of fentanyl using a one-trial behavioral sensitization paradigm. In this experiment, adult and adolescent male and female Sprague-Dawley rats (*n*=7-8) were injected once with fentanyl (200 or 400 µg/kg, sc) or saline and placed immediately in locomotor activity chambers for 60 min. After a 48-h abstinence period, all rats were injected with fentanyl (200 µg/kg, sc) and placed in the locomotor activity chambers for 120 min. On the first injection day (i.e., the pretreatment day), fentanyl reduced the locomotor activity of all groups as rats injected with fentanyl (200 and 400 µg/kg) were less active than rats treated with saline. On the second injection day (i.e., the test day) rats pretreated with fentanyl exhibited more locomotor activity than rats pretreated with saline; however, like on the pretreatment day, fentanyl had a suppressant effect on locomotor activity during the first h. Interestingly, while all rats exhibited a sensitized response, fentanyl-

treated adult male rats were only significantly more active than the controls on the first time block (i.e., the first 10 min). In contrast, adolescent rats and adult female rats treated with fentanyl exhibited increased locomotor activity when compared to saline-treated rats on time block 1 and the last 12 time blocks (i.e., the final 60 min of testing). Regardless of pretreatment condition, adult female rats exhibited more activity than male rats on the test day. Our results suggest that adult female and adolescent rats show an enhanced behavioral response to repeated fentanyl exposure and may be more susceptible to fentanyl use and abuse.

Disclosures: C.A. Crawford: None. **O. Orozco:** None. **H. Diaz:** None. **D.L. Sanchez:** None.

Poster

240. Opioids: Mechanisms of Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 240.09/V27

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH grant R21DA045274

Title: Distributions of kappa opioid receptor (KOR) in paraventricular thalamic nucleus (PVT) and habenula (Hb) in the mouse brain

Authors: *P. HUANG, C. CHEN, L.-Y. LIU-CHEN;
Ctr. for Substance Abuse Res. & Dept. of Pharmacol., Temple Univ. Lewis Katz Sch. of Med., Philadelphia, PA

Abstract: Activation of the KOR *in vivo* produces many affective effects, including dysphoria / aversion and anxiety- and depression-like responses. Accumulating evidence has shown that PVT and Hb may encode both aversive and rewarding aspects of external stimuli. KOR was shown to be present in both PVT and Hb by KOR autoradiography in rats. To investigate KOR distribution at higher resolution, we generated a knockin mouse line that expresses a fusion protein of KOR-tdTomato (KtdT) by homologous recombination. Homozygous KtdT/KtdT mice had intact neuronal circuitry for KOR-mediated behaviors. Immunohistochemistry (IHC) staining of KtdT/KtdT mouse brain sections with antibodies against tdT revealed that the distribution of KtdT was highly similar to that of autoradiography of [^{3}H]U69,593 binding to the KOR. In addition to IHC studies using KtdT/KtdT mice, fluorescence *in situ* hybridization (FISH) studies with RNAscope probes were performed in both KtdT/KtdT and wildtype mice. Moderate and low levels of KtdT and KOR mRNA were observed in PVT and Lateral Hb (LHb), respectively. In both the PVT and LHb, KOR mRNA is localized in a subset of neurons expressing vesicular glutamate transporter 2 (VGLUT2) mRNA. As the mu opioid receptor (MOR) mediates euphoria / reward, in contrast to KOR, and the medial Hb (MHb) expresses a high level of MOR, we will examine distribution of KOR, MOR and VGLUT at the cellular level

in the PVT and Hb by FISH and IHC (when possible). This comparison may shed light on neuroanatomical basis at cellular level by which PVT and Hb play roles in behavioral responses to both aversive and rewarding stimuli. (supported by NIH grant R21DA045274)

Disclosures: P. Huang: None. **C. Chen:** None. **L. Liu-Chen:** None.

Poster

240. Opioids: Mechanisms of Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 240.10/V28

Topic: G.08. Drugs of Abuse and Addiction

Support: UWEC Summer Research Experiences for Undergraduates Grant

Title: Are naltrexone's discriminative stimulus effects mediated by kappa-opioid receptors in rats with chronic, intermittent sucrose access?

Authors: *H. R. STUTT, J. W. ZAJAC, H. M. DORN, A. M. PETREY, C. E. HERZBERG, M. A. MAREK, D. C. JEWETT;
Univ. of Wisconsin Eau Claire, Eau Claire, WI

Abstract: Naltrexone (NTX) is a nonspecific opioid antagonist that blocks mu-, kappa-, and delta-opioid receptors. We have shown that rats given chronic, intermittent sucrose can discriminate 1.0 mg/kg NTX from saline in an operant choice procedure. In the current study, we began to explore the contributions of kappa-opioid function on NTX's discriminative stimulus effects. To examine this possibility, we tested the effects of the kappa-opioid agonist U69,593 in our discrimination procedure. Ten male Sprague-Dawley rats were given 12 hr access to a 25% sucrose solution and trained to discriminate NTX (1.0 mg/kg) from saline. Once discrimination criteria (80% or greater condition-appropriate responding for 8 of 10 consecutive sessions) were reached, testing began. We conducted two different types of generalization tests to examine the potential contribution of kappa-opioid function to NTX's discriminative stimulus effects in our paradigm. Reversal tests were conducted to determine if U69,593 alters existing NTX's discriminative stimulus effects. During these tests, the training dose of NTX was administered and a test session began 15 minutes later. After accurate recognition of NTX, a single dose of U69,593 (0.001 mg/kg - 0.1 mg/kg, s.c.) was administered, and the ability of U69,593 to alter naltrexone's effects was examined 5 to 120 minutes after the U69,593 injection. No alterations in NTX's discriminative stimulus effects were observed during reversal tests. This indicates kappa opioid receptor activation does not alter existing NTX discriminative stimulus effects. In other generalization tests, we used a cumulative dosing procedure to determine if U69,593 alters the ability of NTX to produce its discriminative stimulus effects. Subjects were pretreated with U69,593 (0.0001 mg/kg - 0.1 mg/kg, s.c.) followed by increasing doses of NTX (0.001 mg/kg -

10 mg/kg, s.c.). Injections of NTX continued until response rates were suppressed or 10 mg/kg NTX was administered. The tests revealed individual differences among subjects. In some subjects, U69,593 reduced the potency of NTX to produce its effects. U69,593 did not alter NTX's rate suppressing effects. We observed that prolonged experience with the chronic, intermittent sucrose access increased the potency of NTX to produce discriminative stimulus effects and rate-decreasing effects. During discrimination training, rate-decreasing effects of NTX were observed and subjects could be trained to recognize smaller doses of NTX. These findings indicate that chronic, intermittent sucrose consumption increases the sensitivity to NTX in ways that are similar to chronic administration of opioid agonists.

Disclosures: H.R. Stutt: None. **J.W. Zajac:** None. **H.M. Dorn:** None. **A.M. Petrey:** None. **C.E. Herzberg:** None. **M.A. Marek:** None. **D.C. Jewett:** None.

Poster

240. Opioids: Mechanisms of Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 240.11/V29

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH/NIMHD Grant G12 RR003051
NIH/NIMHD Grant G12 MD007600
NIH/NIMHD Grant 1SC2DA047809-01

Title: Hippocampal and amygdalar BDNF in the extinction of morphine place preference

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Univ. of Puerto Rico Med. Sci. Campus, San Juan, Puerto Rico

Abstract: Opioid abuse has become a worldwide epidemic issue generating an economic burden of $78.5 billion a year in USA. In an effort to manage its spread, the main mechanisms of treatment have been counseling, drug replacement medication and cognitive therapies. However, these clinical approaches are often ineffective and have high rates of relapse. Here we used preclinical models of addiction to understand the molecular mechanisms underlying opioid-seeking behaviors. In previous studies we found that extinction of morphine-induced conditioned place preference (CPP) upregulated Bdnf transcripts in the ventral striatum/nucleus accumbens (VS/NAc) as compared with extinction-deprived animals (Martinez-Rivera et al., SFN 2016). However, the low BDNF expression in the VS/NAc led us to hypothesize that this increase could be originated by VS/NAc afferents expressing high levels of BDNF for the signaling of drug extinction. Here, we use Western blot analyses to determine BDNF expression in the amygdala and hippocampus, both of which are rich in BDNF expression and associated with the extinction of drug-seeking behaviors. Preliminary results revealed that animals showing extinction of

morphine CPP increased BDNF expression of both the hippocampus and amygdala. Interestingly, in the amygdala, BDNF expression also increased in animals showing extinction-like resistance, suggesting that the extinction experience was sufficient to induce BDNF upregulation, and that amygdalar BDNF might be signaling the rate of extinction learning. Further studies will be aimed to measure BDNF in the VS/NAc and pre-frontal cortex, as well as to trace the BDNF-ergic cell populations in the hippocampus and amygdala. Our study highlights the potential molecular impact underlying exposure-based therapies for extinction of drug maladaptive behaviors.

Disclosures: M.E. Lloret Torres: None. **R.N. Ayala Pagan:** None.

Poster

240. Opioids: Mechanisms of Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 240.12/V30

Topic: G.08. Drugs of Abuse and Addiction

Support: Hartwell Foundation Fellowship

Title: Chronic voluntary nicotine consumption increases future concurrent morphine intake and alters nucleus accumbens synapses

Authors: *S. L. WOLFMAN[1], D. J. KALAMARIDES[1], R. S. PATEL[2], J. A. DANI[1]; [1]Neurosci., Univ. of Pennsylvania Perelman Sch. of Medi, Philadelphia, PA; [2]Univ. of Pennsylvania, Philadelphia, PA

Abstract: Opioid addiction is a major public health problem, particularly in Philadelphia. Nicotine addiction also remains a major public health problem in the US and throughout the world, and studies show that 80-90% of people with opioid use disorder are also smokers. Evidence suggests that opioid addiction and nicotine addiction share neurobiological underpinnings, but the mechanisms that explain this co-abuse are unknown. Additionally, most people start smoking in adolescence, a critical developmental period during which even brief nicotine exposure causes long-lasting changes in brain reward pathways. A few studies have investigated the impact of adolescent pre-treatment with nicotine on future addiction-related behaviors, but the effects of continuous nicotine use throughout adolescence and adulthood on opioid addiction have yet to be examined. Here, we use a mouse model of voluntary drug consumption in which nicotine and morphine are delivered in the drinking water. During adolescence, mice are given 24hr access to both nicotine and control solutions, and this nicotine exposure continues through adulthood. As adults, morphine is introduced, allowing mice to voluntarily consume nicotine, morphine, and control solutions. After >4 weeks of morphine exposure, brain slices are taken, and electrophysiological recordings are made in the nucleus

accumbens (NAc), the main output region of the mesolimbic reward circuit. We find that adolescent nicotine consumption leads to persistent, increased morphine intake and impacts intrinsic and synaptic properties of medium spiny neurons (MSNs) in the NAc. These findings may indicate therapeutic targets that are specific to those with comorbid nicotine and opioid use disorders and inform the development of more effective treatments. Future studies will assess sex differences in morphine consumption and electrophysiological changes in the NAc. We will also investigate any cell-type specific changes in MSNs using D1-tomato transgenic mice. Finally, using optogenetic tools, we will dissect plasticity in various NAc inputs that may cause the observed behavioral differences.

Disclosures: S.L. Wolfman: None. **D.J. Kalamarides:** None. **R.S. Patel:** None. **J.A. Dani:** None.

Poster

240. Opioids: Mechanisms of Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 240.13/V31

Topic: G.08. Drugs of Abuse and Addiction

Support: Rutgers Brain Health Institute
Busch Biomedical Grant Program

Title: Pro-inflammatory cytokine modulation following chronic fentanyl self-administration in rats

Authors: *A. N. DAO, N. J. BEACHER, A. W. KUSNECOV, M. O. WEST;
Psychology, Rutgers Univ., Piscataway, NJ

Abstract: Opiate abuse is associated with an increased prevalence of blood borne viruses and opportunistic infections due to specific immunomodulatory effects of opioid drugs that can influence this susceptibility. Fentanyl, a schedule II opioid, has known immunosuppressive effects but little information is available on how chronic self-administration of fentanyl impacts inflammation both in the brain (e.g. microglial responses) and systemically (e.g. cytokine responses). Conflicting evidence from experimenter administered drug and *in vitro* measures of immune activity emphasize the need for a translatable self-administration animal model of drug abuse. In the current project, we use a rat model to examine the effects of voluntary chronic, long-access fentanyl self-administration (SA) on *in vivo* cytokine production and microglial activation in response to the endotoxin lipopolysaccharide (LPS). Our preliminary data suggest the release of the pro-inflammatory cytokine IL-1β is reduced in response to endotoxin (LPS) in rats that self-administer fentanyl, and literature shows this reduction is correlated with reductions in other circulating pro-inflammatory cytokine levels (e.g. TNF-α, IL-6). LPS induces a

vigorous, full-scale systemic inflammatory response resulting in increased expression of these pro-inflammatory cytokines in control rats. Corresponding to these effects, our ongoing research is expected to show that chronic fentanyl SA in Sprague-Dawley rats suppresses cytokine and microglial responses to LPS after a period of abstinence. To test these hypotheses, male rats will intravenously SA fentanyl (Fent/SA group) and control animals (Sal/SA group) will similarly receive access to SA saline for the same daily 6-hour period. Following the SA period, rats will be injected with 1.5mg/Kg LPS after 1 week of abstinence. Blood will be assayed 2 and/or 6 hours after injection of LPS to measure IL-1β, TNF-α, and IL-6. Brains will be collected after perfusion and immunohistochemistry will be used to stain for CD11b to quantitate microglial cell numbers and morphological states. The results of this study may show for the first time that chronic fentanyl SA significantly suppresses the *in vivo* cytokine response to endotoxin challenge with possible implications for opiate relapse.

Disclosures: **A.N. Dao:** None. **N.J. Beacher:** None. **A.W. Kusnecov:** None. **M.O. West:** None.

Poster

240. Opioids: Mechanisms of Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 240.14/V32

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA044766
The John R Murlin Fund

Title: Morphine preference in mice is decreased by fibroblast growth factor 21 (FGF21)

Authors: *L. DORVAL, B. I. KNAPP, J. M. BIDLACK;
Pharmacol. and Physiol., Univ. of Rochester, Rochester, NY

Abstract: Fibroblast growth factors (FGFs) are polypeptides involved in many biological processes such as embryonic development, multiple endocrine signaling pathways, and organogenesis. Unlike most growth factors, fibroblast growth factor 21 (FGF21) crosses the blood-brain barrier. FGF21 binds to a conventional FGF tyrosine kinase receptor in complex with β-Klotho (KLB). Previous studies showed that transgenic mice expressing high levels of FGF21 (FGF21-Tg) had a decreased alcohol and saccharin preference compared to wild-type mice (Talukdar et al, 2016). Mice lacking KLB in neurons had an increased alcohol preference. Furthermore, the effects of FGF21 on taste preference correlated with a decrease in dopamine levels in the nucleus accumbens. Therefore, FGF21 may modulate the effects of opioids in the reward pathway. The goal of this study was to explore the effects of FGF21 on morphine preference via the biased condition place preference (CPP). FGF21-Tg mice express a 2,400-fold

increase in FGF21 protein levels in serum compared to wild type littermates. FGF21-Tg and wild-type littermates were injected with morphine or saline over a 3-day period and placed in the left or right chamber of the CPP apparatus. The initial preference was similar between all mice. However, after the conditioning phase, male transgenic mice spent less time in the morphine associated chamber at 10 mg/kg morphine compared to wild-type male littermates (43% lower preference). Female FGF21-Tg mice showed a 45% lower preference for 10 mg/kg morphine compared to wild-type female littermates. In addition, female transgenic mice showed a 95% lower preference for the morphine-associated chamber at 3 mg/kg morphine compared to wild-type female littermates. These results show that FGF21 reduces morphine preference in mice.

Disclosures: L. Dorval: None. **B.I. Knapp:** None. **J.M. Bidlack:** None.

Poster

240. Opioids: Mechanisms of Dependence

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Program #/Poster #: 240.15/V33

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH training grant T32-DA-028874-07
CMRF grant

Title: Adolescent nicotine alters adult morphine preference and ventral tegmental area inhibitory circuitry in mice

Authors: *D. J. KALAMARIDES, S. L. WOLFMAN, R. S. PATEL, J. A. DANI;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Nicotine dependence has been implicated as a risk factor for opioid dependence and more than 80% of people that misuse opioids also use nicotine. In response to skyrocketing levels of nicotine intake among young people, research must investigate the mechanistic role of nicotine on future opioid sensitivity and understand the nuances of concurrent nicotine and opioid use. To determine neural bases of polysubstance abuse, we used a mouse model of voluntary nicotine consumption during adolescence and voluntary nicotine/morphine co-consumption during adulthood. We then performed patch-clamp electrophysiology experiments to determine if nicotine during adolescence produces lasting changes in reward circuitry. Adult male mice that consumed nicotine in adolescence exhibited increased morphine drinking preference and altered morphine locomotor sensitization compared to controls that only consumed nicotine in adulthood. In slices taken after multiple months of concurrent nicotine and morphine access, we observed a decrease in spontaneous inhibitory postsynaptic current frequency in ventral tegmental area dopamine neurons of mice exposed to nicotine beginning in adolescence relative to mice with nicotine beginning in adulthood. Our voluntary consumption

protocol for polysubstance abuse models the human condition by demonstrating that nicotine impacts future opioid preference. Even after long-term opioid use, nicotine starting in adolescence may persistently alter the mesolimbic dopamine system in a way that impacts later stages of opioid addiction and treatment. Understanding the neurobiological underpinnings of polysubstance abuse will be critical for developing novel therapeutic targets. Future experiments will explore, and aim to manipulate, forms of synaptic plasticity that contribute to the aberrant reward circuitry.

Disclosures: **D.J. Kalamarides:** None. **S.L. Wolfman:** None. **R.S. Patel:** None. **J.A. Dani:** None.

Poster

240. Opioids: Mechanisms of Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 240.16/V34

WITHDRAWN

Poster

240. Opioids: Mechanisms of Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 240.17/V35

Topic: G.08. Drugs of Abuse and Addiction

Support: Engdahl Family Grant, University of Minnesota

Title: Neural mechanisms underlying secondary trauma-induced relapse to opioid seeking in mice

Authors: *J. C. GEWIRTZ[1], T. MATVEEVA[1], A. H. FROST[1], M. T. PISANSKY[2];
[1]Dept. of Psychology, [2]Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: The capacity to experience emotions closely matching those of others is a key component of empathy. However, this process can also be harmful, especially when the distress of another elicits severe distress on the part of the observer. Exposure to stress can result in relapse to drug-seeking behaviors even after long periods of abstinence. We are currently investigating the role of observational distress in relapse to opioid seeking, as well as underlying neural mechanisms, using a mouse model. Male mice were trained in a conditioned place preference (CPP) paradigm, in which one chamber was paired with a morphine injection (15mg/kg), and the opposite chamber with a saline injection. Animals underwent 8 days of conditioning, followed by 14 days of extinction. Mice then observed the distress of a conspecific exposed to a male Sprague-Dawley rat. Fear behaviors (freezing, escape, burrowing, etc.) were recorded. The neuropeptide oxytocin was administered intranasally (20 μg/kg) either acutely or chronically. The oxytocin antagonist L-368,899 hydrochloride was administered systemically (IP; 10 mg/kg). We observed full reinstatement of morphine CPP following exposure to a distressed conspecific. Fear-related behaviors in the observer were exacerbated by oxytocin administration, whereas observational fear and reinstatement of opioid seeking were blocked by administration of an oxytocin antagonist. We are currently investigating the role of endogenous oxytocin in these behaviors using DREADDs combined with fiber photometry in a line of Oxytocin-IRES-Cre/+ mice. Our data suggest that observational fear can induce relapse to opioid-seeking, and establish a key role of oxytocin in mediating this phenomenon. These data have implications for the neurobiology of “secondary trauma” and its interactions with opioid addiction.

Disclosures: **J.C. Gewirtz:** None. **T. Matveeva:** None. **A.H. Frost:** None. **M.T. Pisansky:** None.

Poster

240. Opioids: Mechanisms of Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 240.18/V36

Topic: G.08. Drugs of Abuse and Addiction

Title: Stress interferes with conditioned place aversion induced by morphine in rats

Authors: *Y.-J. LU, A. C. W. HUANG;
Fo Guang Univ., Yilan City, Taiwan

Abstract: As far as we know, stress events might sensitize the brain dopamine reward system and facilitate abused drugs-induced conditioned place preference. In the clinical aspect, patients with drug addiction always suffer from stress in daily life and thereby induce a stronger reward resulting in a severe symptom of abused drugs. This present study focused on this issue that how footshock-induced stress affected conditioned place preference induced by morphine in animal model. During the beginning phase, rats were respectively assigned into no footshock and footshock treatments. The footshock treatment was given 3 mA for 10 seconds in the footshcok box once a trial. Then, rats were given the treatment of conditioning for CPP with morphine injections to pair with a specific compartment of the CPP box for 30 min. The unpaired treatment with normal saline was with another compartment of the CPP box for 30 min. The paired-unpaired regimen was for 10 trials. Finally, the CPP test was performed for 10 min.
The present results indicated that (a). morphine induced conditioned place aversion but not preference. (b). footshock decreased morphine-induced conditioned place aversion. Therefore, footshock-induced stress could reduce morphine-induced conditioned place aversion. The findings might offer some implications in drug addiction for clinical studies.
Keywords: morphine, stress, footshock, conditioned place preference/aversion, drug addiction
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(*) indicated that corresponding author.

Disclosures: Y. Lu: None. **A.C.W. Huang:** None.

Poster

240. Opioids: Mechanisms of Dependence

Location: Hall A

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Program #/Poster #: 240.19/V37

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH/NIDA DP1 DA046537
NIH/NIDA K01 DA039308

Title: Chronic morphine exposure and extended abstinence elicit changes in gene expression in the nucleus accumbens of male and female rats

Authors: *H. L. MAYBERRY[1], D. R. PETERSON[1], S. H. DOWNEY[1], S. BHAKTA[1], A. R. BONGIOVANNI[1], A. S. ELLIS[2], A. B. TOUSSAINT[1], M. E. WIMMER[1];
[1]Temple Univ., Philadelphia, PA; [2]Univ. of Pennsylvania, Philadelphia, PA

Abstract: BACKGROUND
Relapse is a major contributing factor to the current opioid epidemic. Despite years of research, current treatments are not highly effective and relapse rates remain high. Using an incubation of craving model and RNA sequencing, our work aims to elucidate molecular mechanisms underlying cue-induced drug-seeking behavior after morphine self-administration.
METHODS
Male and female Long Evans rats were trained to intravenously self-administer morphine for ten days, controls received saline. After either one or thirty days of forced abstinence, animals were tested for drug-seeking behavior in response to drug-associated cues, or brain tissue was collected and prepared for RNA sequencing. The nucleus accumbens shell, a region facilitating addiction-related behaviors, was analyzed for changes in gene expression, comparing one day and 30 days of abstinence.
RESULTS
In males, differential gene expression analyses comparing saline- to morphine-treated rats after one day of abstinence revealed 76 upregulated genes, and 596 downregulated genes. Comparing day one to day 30 of abstinence in morphine-experienced male animals uncovered changes to 48 genes (36 upregulated, 12 downregulated). Gene ontology and pathway analyses of both gene sets suggest enrichment in genes involved in cell-cell adhesion, DNA regulation of transcription, alternative splicing, protein kinases, and protein phosphorylation. RNA sequencing results from females suggest a distinct pattern of gene expression changes in the nucleus accumbens shell. Although behavioral data indicate that both sexes exhibit incubation of morphine craving following extended abstinence after chronic morphine exposure, the underlying molecular mechanisms of this behavior may diverge based on biological sex.
CONCLUSIONS
Incubation of morphine craving was observed in male and female rats. RNA sequencing of the nucleus accumbens shell revealed differential gene expression between saline controls, and morphine-treated rats in early and late abstinence. Furthermore, gene expression changes related to morphine exposure and abstinence differed between sexes. Future studies will test the functional relevance of these targets in vivo using pharmacological and/or viral gene-targeted interventions. This study lays the groundwork for a more robust understanding of the molecular changes that accompany opioid craving and may contribute to relapse, in order to better inform treatments for opioid relapse.

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Poster

240. Opioids: Mechanisms of Dependence

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH/NIDA K01 DA039308
NIH/NIDA DP1 DA046537

Title: Paternal morphine exposure causes maladaptive behavior in male progeny

Authors: *A. R. BONGIOVANNI[1], A. B. TOUSSAINT[1], A. S. ELLIS[2], S. BHAKTA[1], M. C. KNOUSE[1], A. S. THOMAS[2], H. MAYBERRY[1], L.-Y. LIU-CHEN[3], M. E. WIMMER[1]; [1]Dept. of Psychology and Neurosci., Temple Univ., Philadelphia, PA; [2]Dept. of Psychiatry, Univ. of Pennsylvania, Philadelphia, PA; [3]Ctr. for Substance Abuse Res., Temple Univ. Lewis Katz Sch. of Med., Philadelphia, PA

Abstract: A growing body of literature indicates that parental environmental insults can influence the behavior and biology of offspring. According to recent estimates, 5 million children have fathers afflicted by substance abuse. Furthermore, consistent preclinical and clinical evidence suggests that parental drug exposure can have deleterious consequences for progeny. Considering the opioid addiction crisis, understanding the long-term impacts of parental drug-exposure on subsequent generations is essential. Here, we sought to determine the influence of chronic paternal morphine self-administration on the behavior of first generation (F1) progeny. Male rats self-administered morphine (0.75mg/kg/infusion) for 60 days (the duration of rat spermatogenesis); controls received saline. Following this chronic regimen of morphine or saline self-administration, sires were bred with drug-naïve females to produce F1 offspring. The adult (60-90 days) male and female F1 progeny of morphine-exposed and saline-treated sires were behaviorally tested for addiction-like traits. F1 offspring were allowed to self-administer morphine (0.25mg/kg/infusion) for 10-days on a FR1 reinforcement schedule. We found that male, but not female, offspring took more morphine than their respective controls. F1 morphine-sired male offspring also worked harder to receive infusions of morphine under a progressive ratio schedule. This phenotype seemed to be drug-specific, in that sucrose and cocaine self-administration were not altered by paternal morphine history in male or female F1 progeny. Together, these results suggest that the reinforcing efficacy of morphine is enhanced in male offspring of morphine-exposed sires. We intend to use this multigenerational model to

identify the underlying mechanisms of opioid addiction susceptibility to uncover potentially novel therapeutic targets.

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Poster

240. Opioids: Mechanisms of Dependence

Location: Hall A

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Support: NIH/NIDA grant R21 DA037728 (Gewirtz, JC and Harris, AC, Co-PIs)
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Grant in Aid of Opioid Research, University of Minnesota

Title: Withdrawal-induced anhedonia after opioid exposure: Searching for molecular signals that predict subsequent opioid self-administration

Authors: *Y. SWAIN[1,4], S. X. LIU[1], M. GADES[1], P. MUELKEN[4], M. G. LESAGE[1,4,2], P. V. TRAN[3], J. C. GEWIRTZ[1], A. C. HARRIS[1,4];
[1]Psychology, [2]Med., [3]Dept. of Pediatrics, Univ. of Minnesota Twin Cities, Minneapolis, MN; [4]Hennepin Healthcare Res. Inst., Minneapolis, MN

Abstract: Initial exposure to opioid and other drugs produces affective withdrawal signs including anhedonia ("acute dependence"). We are interested in whether the intensity of acute dependence is predictive of the severity of subsequent drug use and in investigating molecular mechanisms underlying any such association. In Study 1, rats were first tested for withdrawal-induced anhedonia (WIA) using intracranial self-stimulation (ICSS) and somatic signs during naloxone-precipitated and spontaneous withdrawal from acute injections of morphine. They were then tested for addiction vulnerability using various measures of morphine self-administration (MSA) including acquisition, essential value, and morphine- and stress-induced reinstatement. After MSA, withdrawal was assessed again ("late-stage dependence"). Greater naloxone-precipitated and spontaneous WIA from initial morphine exposure was associated with lower vulnerability on multiple MSA measures. In contrast, WIA during late stage dependence and

somatic signs during either acute dependence or late-stage dependence did not correlate with any MSA measure. These data indicate that high anhedonia during withdrawal from initial opioid exposure uniquely predicts lower subsequent opioid use in rats. In Study 2, we conducted RNA-seq followed by qPCR verification on tissue collected from the prefrontal cortex of male and female rats 23 hrs following a similar regimen of morphine injections as was used to produce anhedonia in Study 1. Expression of 529 genes was significantly affected, with an overlap between males and females of 34%. Of this subset, enrichment was greatest in genes associated with cell death/survival, cell signaling, cell morphology, cellular development, and cellular assembly/organization. Current and future efforts are directed towards identifying which of these transcriptomic changes are critically important in conferring resilience to opioid addiction and establishing the role of WIA as an endophenotype of opioid addiction vulnerability.

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Poster

240. Opioids: Mechanisms of Dependence

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Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R01DA045000

Title: Characterization of oxycodone self-administration and withdrawal-associated negative affect in male and female rats

Authors: *S. K. GUHA, N. J. CONSTANTINO, E. H. CHARTOFF;
McLean Hospital, Harvard Med. Sch., Belmont, MA

Abstract: Opioid Use Disorder (OUD) is characterized by initial abuse, transition to impulsive-compulsive behavior, and emergence of withdrawal-associated long-lasting affective disorder and subsequent relapse. The closing gender gap in OUD highlights the importance of understanding its progression and neurobiological substrates in males and females. The aim of this study is to use a rat model of prescription OUD: oxycodone self-administration (SA) to delineate putative sex differences in acquisition and escalation of drug-taking; abstinence induced withdrawal signs; and incubation of craving as measured by post-abstinence cue-induced drug-seeking behavior. Adult male and female Sprague-Dawley rats learned to self-administer 0.06 mg/kg of oxycodone per infusion on an FR1 schedule of reinforcement for 8 d of 1-h short-access, followed by 14 d of 6-h long-access (LgA) and 14 d of abstinence. On abstinence day 15, rats underwent 2 h of saline SA in the previously drug-paired chamber. To monitor motivational state and reward sensitivity in each rat throughout the different addiction-

like phases, intracranial self-stimulation (ICSS) was conducted 16-h after each days' SA session (1-h prior to the next days' SA session), and in a separate cohort, 2-h after each days' SA session. Both male and female rats readily acquired oxycodone SA and escalated drug intake during the LgA regimen. Pattern analysis showed that rats moved to a periodic pattern of drug intake that became more entrained in later phase of LgA oxycodone SA. Upon re-exposure to operant chambers previously paired with oxycodone SA after 14 d of abstinence, male and female rats reinstated drug-seeking. ICSS stimulation thresholds measured 16-h and 2-h post oxycodone SA as a measure of anhedonia. Our results showed that 2-h post oxycodone SA, there was a trend towards increase in ICSS threshold in males, suggesting an anhedonia-like response. Interestingly, while females did not definitively exhibit anhedonia-like response, post 14-d abstinence, female rats exhibited an increased incubation of oxycodone craving. Our results provide a nuanced characterization of oxycodone-intake in males and females during both short and long access periods that are thought to model the transition from abuse to OUD. These early results exhibited an interesting sexual dimorphism where male rats showed anhedonia due to drug administration, while female rats exhibited an increased incubation of craving post 14-d abstinence, and underlining the need to study sexually dimorphic responses to opioid addiction

Disclosures: S.K. Guha: None. **N.J. Constantino:** None. **E.H. Chartoff:** None.

Poster

240. Opioids: Mechanisms of Dependence

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NIH Grant AA026999

Title: Targeting the orexin system for prescription opioid use disorder: Efficacy of orexin-1 receptor antagonist SB334867 in preventing oxycodone taking and seeking in rats

Authors: *A. MATZEU, R. MARTIN-FARDON;
Neurosci., The Scripps Res. Inst., La Jolla, CA

Abstract: Opioid abuse and overdose have risen to epidemic proportions in the United States in recent years. Prescription opioids, such as oxycodone, are potent analgesics that are used to treat and manage pain. Oxycodone is one of the most commonly abused prescription drugs. Finding an effective strategy to prevent prescription opioid use disorder is urgent. Orexin receptors

(OrxR1 and OrxR2) have been proposed as potential targets for anti-craving medications. OrxR1 and OrxR2 have been implicated in the regulation of motivation, especially highly motivated behavior, arousal, and stress, making them ideal targets for the treatment of addiction. Therefore, this study tested whether OrxRs blockade prevents excessive oxycodone intake and relapse. Male Wistar rats were made dependent by voluntary lever pressing for oxycodone (0.15 mg/kg/infusion, i.v.) 12 h/day for 21 days. Using this procedure, the rats progressively escalated their oxycodone self-administration over the 21 days of training and exhibited significant physical signs of opioid withdrawal that began on day 15. After training, the effects of the OrxR1 antagonist SB334867 (0-30 mg/kg, i.p.) and OrxR2 antagonist TCSOX229 (0-30 mg/kg, i.p.) on oxycodone self-administration were tested. SB334867 at 10 and 30 mg/kg significantly decreased oxycodone self-administration, whereas TCSOX229 did not produce any effects at any of the doses tested. To investigate whether OrxR1 blockade also prevents oxycodone-seeking (relapse) behavior, a separate group of male Wistar rats was trained to self-administer oxycodone (12 h/day for 21 days) in the presence of a contextual/discriminative stimulus (S^D). The rats then underwent 2-h daily extinction training, during which oxycodone and the S^D were withheld. After extinction, the ability of SB334867 (0-30 mg/kg) to prevent S^D- induced conditioned reinstatement of oxycodone-seeking behavior was tested. At the highest dose tested (30 mg/kg), SB334867 decreased oxycodone-seeking behavior, suggesting that targeting OrxR1 may prevent prescription opioid craving and relapse. These results suggest that OrxR1 antagonism prevents excessive prescription opioid use, craving, and relapse. The results support the hypothesis that targeting OrxR1 might be beneficial for the treatment of prescription opioid use disorder.

Disclosures: A. Matzeu: None. **R. Martin-Fardon:** None.

Poster

240. Opioids: Mechanisms of Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 240.24/V42

Topic: G.08. Drugs of Abuse and Addiction

Support: R01-DA037426 (MMR)
F31-DA042502 (SS)

Title: Chronic morphine-induced changes in gene expression within dopaminergic ventral tegmental area neurons

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Abstract: Opiate abuse is a growing epidemic in the US and has led to a large increase in overdose deaths. Despite the significant risk for abuse that opiates possess, much is still unknown about the neuroadaptations that occur with chronic use. To date, many studies have focused on opiate-induced changes in mesocorticolimbic reward circuit function. For example, chronic administration of opiates, such as morphine, is known to alter activity and morphology of dopamine (DA) neurons within the ventral tegmental area (VTA). Our lab is interested in identifying the molecular mechanisms underlying these changes in VTA DA structure and function. However, large-scale gene expression studies have been limited to homogenization of the entire VTA, which includes both GABAergic and dopaminergic neurons, potentially obscuring changes that occur specifically in VTA DA neurons. We sought to address this knowledge gap through the use of using Translating Ribosome Affinity Purification (TRAP) to extract actively translating mRNA specifically from VTA DA cells. Specifically, we crossed L10-EGFP and DAT-Cre mice to label RNA in DA cells and then subcutaneously implanted mice with sham or morphine pellets. We then performed RNA sequencing analysis on pooled VTA samples. We first confirmed that our samples were enriched for DA transcripts and depleted of GABA, glutamatergic and glial transcripts. Excitingly, we identified a number of genes whose expression specifically in VTA DA cells were significantly impacted by morphine administration. We have validated morphine-induced changes in gene expression via RT-PCR of a number of candidate genes including neuropeptides, ion channels, and regulators of cytoskeletal remodeling. We are currently working on interrogating the functional impact of a subset of these novel gene targets, as well as defining their expression pattern within the VTA. In conclusion, this work seeks to define the changes in the VTA DA transcriptome induced by morphine in order to identify potential novel therapeutic targets for the treatment of opiate addiction.

Disclosures: A.L. Garrison: None. **S.E.C. Simmons:** None. **E.A. Heller:** None. **Q. Hu:** None. **M.S. Mazei-Robison:** None.

Poster

240. Opioids: Mechanisms of Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 240.25/V43

Topic: G.08. Drugs of Abuse and Addiction

Support: T32 NS044928-13, NINDS
PhRMA Foundation Research Starter Grant
R01 DA039895-01A1, NIDA

Title: SGK1 activity in VTA dopamine neurons regulates cocaine and morphine reward behaviors

Authors: *M. A. DOYLE[1], V. BALI[2], E. S. WILLIAMS[3], A. R. STARK[4], A. J. ROBISON[2], M. S. MAZEI-ROBISON[2];
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Abstract: Drugs of abuse are known to regulate activity of the mesolimbic dopamine (DA) system. Specifically, drug-induced changes in ventral tegmental area (VTA) cellular activity and gene regulation contribute to behavioral outputs associated with addiction. Our previous work has determined that serum- and glucocorticoid-inducible kinase 1 (SGK1) catalytic activity is increased by chronic, but not acute, administration of cocaine or morphine. Furthermore, I have shown that viral overexpression of SGK1 mutants in the VTA of adult mice produce behaviorally relevant effects on drug reward, assessed by cocaine conditioned place preference (CPP) and voluntary morphine intake using a two-bottle choice task. Specifically, intra-VTA infusion of a catalytically inactive SGK1 mutant (K127Q) significantly decreases cocaine CPP and morphine preference, suggesting that decreasing VTA SGK1 activity is sufficient to decrease drug reward. To more fully understand the role of VTA SGK1 in behaviors relevant to addiction, I am now manipulating SGK1 expression in a cell type-specific manner to determine whether SGK1 activity in DA or GABA neurons drives the observed behavioral effects. Utilizing novel Cre-dependent viral constructs, I have found that decreased SGK1 activity in VTA DA neurons significantly decreases cocaine CPP, while preliminarily data suggests that this same manipulation in VTA GABA neurons has no effect. Future studies look to determine a potential mechanism for these behavioral effects using ex vivo slice electrophysiology, and parallel studies currently explore the potential effects of a similarly regulated SGK1 phosphorylation site (Ser78) in drug-related behaviors. These studies will allow for identification of the specific cells and circuits that are critical for SGK1-mediated effects on drug reward and intake. Altogether, this work will increase our understanding of the role of VTA SGK1 activity in drug-related behaviors, a necessary step in assessing the feasibility of SGK1 inhibition as a novel therapeutic avenue for addiction.

Disclosures: M.A. Doyle: None. **V. Bali:** None. **E.S. Williams:** None. **A.R. Stark:** None. **A.J. Robison:** None. **M.S. Mazei-Robison:** None.

Poster

240. Opioids: Mechanisms of Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 240.26/V44

Topic: G.08. Drugs of Abuse and Addiction

Support: R01 DA039895-01A1, NIDA
Genevieve Gillette Fellowship

Title: Use of fentanyl to investigate regulation of drug reward by VTA SGK1

Authors: *A. R. STARK[1], M. A. DOYLE[1], M. S. MAZEI-ROBISON[2];
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Abstract: According to the National Institute on Drug Abuse, over 130 people per day die of an opioid overdose, indicative of a major health problem in the U.S. Opioid abuse results in part from neuroadaptations in the mesocorticolimbic reward circuit, specifically in the ventral tegmental area (VTA). Our lab is studying the role of the protein serum- and glucocorticoid-regulated kinase 1 (SGK1) in the VTA in regulating drug reward. We have previously found that chronic morphine administration increases VTA SGK1 phosphorylation and catalytic activity, and, furthermore, VTA injection of a catalytically inactive version of SGK1 (HSV-SGK1-K127Q) reduces opioid reward behavior in the morphine two-bottle choice test (TBC). However, the necessary use of quinine as a bitter taste control in the morphine TBC test presents a confounding variable, as quinine on its own is aversive. One strategy to reduce this potentially confounding effect is the use of fentanyl, another opioid 50-100 times stronger than morphine. Due to its increased potency, much lower concentrations of fentanyl could be used in the TBC test, eliminating bitter taste as a confounding variable. In order to test this, I will run male and female mice through a single fentanyl TBC test with escalating doses in order to establish a dose response curve. In parallel, I will perform biochemical assays to confirm that chronic fentanyl induces VTA SGK1 phosphorylation and catalytic activity similar to morphine. Specifically, I will perform intraperitoneal (IP) injections of fentanyl in male and female mice for seven days. Mice will then be sacrificed and VTA tissue will be processed for Western blot analysis. Given the robust increase in VTA SGK1 biochemistry by morphine and the similar actions of morphine and fentanyl in the mesocorticolimbic reward circuit, I predict that chronic fentanyl will induce increases in VTA SGK1 phosphorylation and catalytic activity. Following validation experiments, I plan to run mice with viral manipulations of SGK1 through a fentanyl TBC test in order to evaluate the effects of VTA SGK1 activity on fentanyl reward. I predict that overexpression of a catalytically inactive version of SGK1 (K127Q) in the VTA will decrease fentanyl preference, as fentanyl and morphine both act as opioid receptor agonists. The results of this project will expand our knowledge of SGK1's involvement in opioid reward behaviors and could present SGK1 as a potential therapeutic target for opioid addiction treatments.

Disclosures: A.R. Stark: None. **M.A. Doyle:** None. **M.S. Mazei-Robison:** None.

Poster

240. Opioids: Mechanisms of Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 240.27/V45

Topic: G.08. Drugs of Abuse and Addiction

Support: DA08259

Title: Oxycodone injections not paired with conditioned place preference have little effect on the hippocampal opioid system in female and male rats

Authors: *E. ASHIROVA[1], N. H. CONTOREGGI[1], M. A. JOHNSON[1], F. J. AL-KHAYAT[2], G. A. CALCANO[1], B. REICH[1], E. M. O'CINNEIDE[3], Y. ZHANG[4], Y. ZHOU[4], B. S. MCEWEN[3], M. KREEK[4], T. A. MILNER[1];
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Abstract: Following oxycodone conditioned place preference (CPP), Leu-enkephalins (LEnk) and mu and delta opioid receptors (MORs and DORs) redistribute in hippocampal circuits in a manner that facilitates opioid-associative learning processes, particularly in female rats. Here, we examined if oxycodone injections that are not paired with CPP similarly affect the opioid system. Naïve rats were injected with oxycodone (3mg/kg, I.P.) or saline using the same 14-day timeline as the CPP paradigm, but without being placed in the behavioral apparatus. Rat brains were perfusion fixed with aldehyde solutions and then sections were: 1) immunolabeled by light microscopy with LEnk or 2) dual labeled with GABA (or parvalbumin) using immunoperoxidase and DOR (or MOR) using silver-intensified gold (SIG) and processed for electron microscopy. **LEnk:** No differences in LEnk levels in the mossy fiber pathway were seen in either the CA3 or dentate gyrus in females and males injected with oxycodone or saline. **DORs:** In CA3 stratum radiatum dendrites, there were no differences in the densities of DOR-SIG particles between any of the four injected groups. DOR-SIG densities were elevated in dentate hilar GABAergic dendrites of Saline-injected females compared to Saline-injected males; however, there was no change in these DOR-SIG densities following oxycodone injections. **MORs:**Saline-injected females compared saline-injected males had higher MOR-SIG densities on and near the plasmalemma and in the cytoplasm of parvalbumin-containing dendrites in the dentate hilus. Plasmalemmal MOR-SIGs on hilar parvalbumin dendrites decreased in oxycodone-injected females compared to saline-injected females, which would reduce disinhibition (i.e., excitation) of granule cells. The distribution of MOR-SIGs in parvalbumin dendrites was unaltered in males following oxycodone injections. These results indicate that changes in the hippocampal opioid system that would promote opioid-associative learning processes do not occur with administration of oxycodone alone, and instead must be paired with CPP.

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Poster

240. Opioids: Mechanisms of Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 240.28/V46

Topic: G.08. Drugs of Abuse and Addiction

Support: DA08259

Title: Sex and chronic stress differentially alter phosphorylated mu and delta opioid receptor levels in the rat hippocampus following oxycodone conditioned place preference

Authors: *J. R. BELLAMY[1], B. REICH[1], A. ZVEROVICH[1], Y. ZHOU[2], N. H. CONTOREGGI[1], J. D. GRAY[3], B. S. MCEWEN[3], M. J. KREEK[2], T. A. MILNER[1,3];
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Abstract: Following oxycodone conditioned place preference (CPP) in naïve female and male Sprague Dawley rats, delta- and mu-opioid receptors (DORs and MORs) redistribute in hippocampal CA3 pyramidal cells and GABAergic interneurons in a manner that would promote learning processes particularly in females (Ryan et al., 2018). In females, but not males, MORs and DORs similarly redistribute in CA3 and hilar neurons following chronic immobilization stress (CIS), essentially "priming" the opioid system for oxycodone-associative learning (Reich et al., 2019). Following CIS, only females acquire oxycodone CPP. The present study determined whether sex and CIS differentially affect the levels of phosphorylated MORs and DORs (pMORs and pDORs, respectively) in the hippocampus following oxycodone CPP. In naïve rats, the density of pMOR-immunoreactivity (ir) and pDOR-ir in the CA1 and CA3 regions were similar in Saline-injected (Sal)-females and Sal-males. However, pMOR-ir was increased in CA1 stratum oriens and CA3b strata lucidum and radiatum in oxycodone-injected (Oxy)-females compared to Sal-females. Additionally, the density of pDOR-ir increased in the pyramidal cell layer and stratum radiatum of CA2/3a in Oxy-males compared to Sal-males. Like naïve rats, the density of pMOR-ir and pDOR-ir in the CA1 and CA3 regions was similar between CIS Sal-females and CIS Sal-males. However, the density of pDOR-ir in the CA2/3a increased in CIS Oxy-females compared to CIS Sal-females. Unlike naïve rats, no changes in the density of either pMOR-ir or pDOR-ir were seen in the hippocampi of CIS Oxy-males compared to CIS Sal-males. Consistent with our prior studies, these findings suggest that oxycodone CPP activates MORs and DORs in select hippocampal circuits. However, the circuit altered following oxycodone CPP varies with sex and CIS. Moreover, the absence of activation of opioid receptors in Oxy-males may contribute to the inability to acquire CPP.

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Poster

240. Opioids: Mechanisms of Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 240.29/W1

Topic: G.08. Drugs of Abuse and Addiction

Support: DA08259

Title: Sex differences in the mRNA expression of opioid peptides and receptors in the rat hippocampus

Authors: *M. A. JOHNSON[1], N. H. CONTOREGGI[2], J. KOGAN[4], M. BRYSON[5], B. REICH[3], J. D. GRAY[6], M. KREEK[7], B. S. MCEWEN[8], T. A. MILNER[2];
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Abstract: Sex and chronic immobilization stress (CIS) can alter mossy fiber Leu-enkephalin levels and the distribution of mu and delta opioid receptors within hippocampal interneurons of Sprague Dawley rats. Notably, sex-dependent changes in opioid-associated genes and receptors in the hippocampus following CIS are paralleled with an inability for males, but not females, to acquire conditioned place preference (CPP) to mu-agonist oxycodone. Here, RNAScope *in situ* hybridization was used to determine if the expression of hippocampal opioid peptides and receptors is altered in unstressed (US) and CIS estrus female and male rats. ***Penk*:** *Penk* expression was elevated in dentate gyrus (DG) granule cells in CIS females and in scattered hilar neurons in CIS females and males compared to US counterparts. In contrast, *Penk* expression was decreased in CA3b interneurons in CIS males compared to US males. ***Oprm1:*** *Oprm1* expression was higher in US females compared to US males in all hippocampal subregions (CA1; CA3a,b; DG). Following CIS, *Oprm1* expression decreased in hilar and CA3b interneurons in CIS females, though the expression levels were still higher than US and CIS males. Additionally, *Oprm1* expression decreased in CA2/CA3a pyramidal cells in CIS males compared to US males. These results are consistent with prior studies which show that females compared to males have higher baseline levels of *Oprm1* expression, making them more sensitive to opioids in hippocampal circuits. ***Oprd1***: *Oprd1* expression was lower in CA3a pyramidal cells in US females compared to US males. Following CIS, *Oprd1* expression

increased in DG interneurons but decreased in CA1 interneurons in both females and males compared to US rats. In CA3a pyramidal cells, *Oprd1* expression increased in CIS females to levels similar to those observed in males. In CA3b interneurons, *Oprd1* expression increased in CIS males compared to US males. ***Oprk1:*** Few interneurons expressed *Oprk1*. However, the number of hilar interneurons expressing *Oprk1* increased in females and males after CIS. The sex- dependent changes in opioid gene expression seen observed following CIS, especially those in CA3a and b, may contribute to the attenuation of oxycodone CPP in males exposed to CIS.

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Poster

240. Opioids: Mechanisms of Dependence

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 240.30/W2

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA Grant No. 05010

Title: Mouse FMRI reveals mu opioid receptor-mediated effects of buprenorphine on whole brain functional connectivity

Authors: *E. DARCQ[1], M. D. T. NASSEEF[1], L. WELSCH[1], J. PUNEET SINGH[1], B. L. KIEFFER[2];
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Abstract: Misuse of mu opioid receptor (MOR) drugs may cause addiction and is a leading cause to the rising opioid epidemic in North America. However, among clinically approved opioids a safer option to treat pain may be buprenorphine, which stands apart due to its lower abuse liability. Buprenorphine exerts its actions through binding to opioid receptors, with a main effect on MOR (partial agonism), but also inverse agonism at the kappa opioid receptor, as well as some activity at delta opioid and NOP receptors. Using resting-state functional magnetic resonance imaging (fMRI) in control and MOR knockout (KO) mice, we recently determined that oxycodone reduces functional connectivity (FC) in a MOR-dependent manner and established a whole-brain FC signature for this notoriously overprescribed MOR agonist. Using this novel approach, we here examined FC alterations elicited by buprenorphine. We tested the effect of an acute buprenorphine administration in both control (CTL) and MOR-KO mice in order to characterize MOR-dependent effects and determine whether effects mediated by other receptors are detectable. MRI was performed on male CTL and MOR-KO mice under anesthesia. FMRI images were acquired using a 7 Tesla scanner and a Cryoprobe with EPI sequence. An

analgesic buprenorphine dose (1,25 mg/kg) was injected inside the scanner ten minutes after an initial baseline scan, which was pursued for another 20 minutes. After pre-processing, pre- versus post-drug functional data were compared for both CTL and MOR KO groups. Our preliminary data indicate that, contrary to oxycodone, buprenorphine modifies FC in the two groups, suggesting that this MOR agonist acts at MOR and other receptors. Focusing on MOR-mediated effects, we found that buprenorphine decreases FC for nucleus accumbens, habenula and periaqueductal gray seeds (t-test, $p<0.05$, cluster correction), and detailed mapping will be shown.

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Poster

241. Decision Making: Rodent Medial Prefrontal Cortex

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 241.01/W3

Topic: H.01. Animal Cognition and Behavior

Support: KAKENHI JP 16K15644

Title: Optogenetically induced neuronal activity mimicking epileptic discharges from hypothalamus to mediodorsal nucleus of thalamus and to lateral habenula deteriorates performance of working memory tasks

Authors: *M. SONODA[1,2,3], H. AIMI[2], K. KAWASAKI[2], H. TODA[2,4], S. HIRAI[5], R. MEGURO[6], M. HORIE[7], H. OKADO[5], S. KAMEYAMA[8], T. YAMAMOTO[3], I. HASEGAWA[2];
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Abstract: Hypothalamic hamartoma (HH) is a rare congenital brain lesion with intractable gelastic seizures (GS) as a major charactaristics. About half of HH patients exhibit cognitive and behavioral impairments called epileptic encephalopathy, whose underlying pathophysiological mechanisms remains undetermined. Previous studies with subtraction ictal SPECT coregistered to MRI (SISCOM) and EEG-fMRI suggested increased activity in the medio-dorsal thalamus (MD)/ lateral habenula (LHb) as a key nucleus of epileptic circuits of GS with HH. We reported that neuropsychological performance significantly improved after disconnective surgery between

HH and normal brain. In the present study, we hypothesized that epileptic discharges from HH to MD/LHb deteriorate cognitive functions related to the prefrontal cortex, which has rich connections from MD/LHb. To test the hypothesis, we induced pathway-specific high-frequency neural activity mimicking epileptic discharges from HH by using an optogenetic approach with channelrhodpsin2 (ChR2) in rats, and examined changes in the performance of a working memory task, the most representative behavioral test of the prefrontal functions. Specifically, we injected pCAG-ChR2-GFP incorporating adeno-associated virus serotype 9 (AAV9) into the lateral hypothalamus area (LHA) and histologically observed GFP expression at the injected area and ipsilateral MD/LHb. We then photostimulated the MD/LHb while recording neuronal activity from LHA, MD/LHb and bilateral prelimbic cortex. Head-fixed rats learned, within 3 months, to perform a working memory task in which the rats were required to pull the appropriate side of spout-lever with their forelimb after 0-9 seconds following the presentation of a visual cue. The performance of the working memory task was selectively impaired by the optogenetically induced abnormal neural activity in the instruction period, not in the delay period. These findings support our hypothesis and suggest that epileptic discharges propagating from HH to MD/ LHb cause the cognitive symptoms observed in epileptic encephalopathy associated with HH.

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Poster

241. Decision Making: Rodent Medial Prefrontal Cortex

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 241.02/W4

Topic: H.01. Animal Cognition and Behavior

Support: FRSG Award from American University

Title: Impact of reward probability and reversal criterion on two-armed bandit performance

Authors: *T. K. SWANSON[1], B. B. AVERBECK[2], M. LAUBACH[1];
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Abstract: Two-armed bandit tasks (TAB; aka probabilistic reversal learning) are used to assess the neuronal basis of cognitive flexibility. TAB tasks are carried out using either blocked- or performance-based reversals in reward probabilities. We developed a normative model of the correlated TAB task based on win-stay/lose-shift (WSLS) rules. The model revealed differences in the roles of WS strategies (response to positive feedback) and LS strategies (response to negative feedback) in determining choice accuracy and number of reversals. We validated the

model by training rats to perform a spatial TAB task. We then tested them on two series of experiments to investigate whether a change in task parameters would lead to a change in strategy. The first series maintained a blocked design but alternated between deterministic and probabilistic (80/20) reward. Strategy did not change despite a decrease in accuracy when outcomes became uncertain. In contrast, when reward outcome shifted from probabilistic to deterministic, both WS and LS behavior increased with a corresponding increase in accuracy. However, accuracy increased over time in control sessions that had stable reward schedules. The second series maintained 80/20 reward likelihoods and shifted between reversal criteria. Shifting from performance-based to blocked reversals led to an increase in correct WS behavior that was not accompanied by a change in accuracy. Shifting from blocked to performance-based reversals led to an increase in incorrect WS behavior which decreased accuracy. Control sessions that used one reversal criterion showed a decrease in accuracy over time. For both experiments, the relationship between strategy and accuracy and number of reversals followed the predictions made by the model. Together, these experiments show that rodents are able to adapt their strategies to account for changes in the environment. Additionally, a subgroup of animals were tested in a blocked task after administration of systemic yohimbine (2mg/kg), which led to a decrease in WS behavior and a corresponding decrease in accuracy. There was also a decrease in learning rate, especially at the beginning of the session. This suggests that feedback processing in the TAB is susceptible to modulation of the noradrenaline system.

Disclosures: T.K. Swanson: None. **B.B. Averbeck:** None. **M. Laubach:** None.

Poster

241. Decision Making: Rodent Medial Prefrontal Cortex

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 241.03/W5

Topic: H.01. Animal Cognition and Behavior

Support: FRSG award from American University

Title: Learning to choose: Rats deliberate in a test of the sequential choice model

Authors: *M. LAUBACH[1], M. W. PRESTON, JR[2], S. R. WHITE[1];
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Abstract: Studies of decision making assume that participants deliberate over their options before making a choice. However, some studies of foraging decisions, mostly done in starlings, have argued that options are processed independently and animals do not deliberate. These studies led to a theory called the “sequential choice model” (Kacelnik et al., 2011). A unique aspect of these studies was how the animals were trained and tested. They were trained by

receiving sequential presentations of individual stimuli associated with different reward values. Choices between stimuli were evaluated only after the animals were extensively trained. We used this training procedure in a standard rodent two-alternative forced-choice design with visual stimuli. Adult male Long-Evans rats initiated trials by responding in a central port. Dynamic visual stimuli with differential luminance were then presented from LED grids located above two adjacent choice ports. Entering a choice port produced fluid from a reward port on the opposite wall in the arena. Luminance was paired with sucrose concentration: 8 randomly moving LEDs -> 16% sucrose; 2 randomly moving LEDs -> 4% sucrose. Rats were trained to respond to single visual stimuli (forced trials) over ten one-hour daily training sessions (>300 trials per session by day 10). Then, the rats experienced test sessions with both visual stimuli presented on one-third of trials (choice trials). Rats showed evidence for deliberation: Response latencies were much shorter (by more than 100 ms) when rats responded to the higher value stimuli on choice trials, as fast as they responded to the lower value stimuli on forced trials. Repeated experience in making choices reduced these latency differences, but rats still showed evidence for deliberation after experiencing five test sessions. To understand the mechanisms of these effects, we used exGauss modeling (Van Zandt, 2000), the fast-DM model (Voss and Voss, 2007), and the robust-EZ diffusion model (Wagenmakers et al., 2008). We found that the exponential decay parameter (Tau) in the exGauss model was always higher on choice trials compared to forced trials and that experience in making choices reduced the mean of the Gaussian parameter (Mu). Furthermore, the mean drift rate in the robust-EZ model was higher on forced trials compared to choice trials (robust-EZ model) and boundary separation decreased with experience in making choices (robust-EZ and fast-DM models). Together, our findings suggest that rats deliberate when choosing between visual stimuli. Furthermore, experience with making choices reduces the extent, and may change the mechanisms, of deliberation.

Disclosures: **M. Laubach:** None. **M.W. Preston:** None. **S.R. White:** None.

Poster

241. Decision Making: Rodent Medial Prefrontal Cortex

Location: Hall A

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Program #/Poster #: 241.04/W6

Topic: H.01. Animal Cognition and Behavior

Support: AA023786
P60-AA007611

Title: Optogenetic inhibition of rat medial prefrontal cortex increases measures of impulsivity during delay discounting

Authors: *S. M. WHITE, C. C. LAPISH, C. L. CZACHOWSKI;
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Abstract: Impulsivity, or the tendency to act prematurely without foresight, has been linked to a diverse range of pathological conditions. Foresight refers to the ability to envision future rewards and events (i.e. prospectively sample) and has been associated with decreased impulsivity. One form of impulsivity is measured by the ability to delay gratification and is often studied in the framework of Delay Discounting (DD). DD provides the means to study impulsivity in a number of pathological conditions. It is not fully understood whether impulsivity results from or precedes development of the pathological state itself. This necessitates an understanding of neurobiological mechanisms contributing to decision making.
Animal models allow invasive techniques to be used to dissect the neurocircuitry involved in decision making. Given that the decision-making process is ongoing rather than an isolated event, optogenetics provide the temporal and spatial specificity necessary for evaluating brain-region-specific contributions to decision making during DD. The present study used optogenetics to assess the role of mPFC in an adjusting-amount DD procedure (n=8). Behavioral measures reflecting the planning of choices as well as impulsivity were assessed. Optogenetic inhibition of mPFC in Wistar rats occurred during discrete Epochs to assess the influence of mPFC on planning behavior throughout the decision-making process. Epoch 1 inactivation occurred from trial start to initiation, while Epoch 2 inactivation occurred beginning initiation and ended once a choice was made.
Inhibiting mPFC during Epoch 1 and Epoch 2 condition increased measures of impulsivity in comparison to a "No Inactivation" ($p<.05$ and $p<.01$, *respectfully*). These data indicate that mPFC plays a critical role in mitigating impulsive responses during DD. Further analyses revealed that "No Inactivation" exhibited a negative association between both choice latency and planning behavior (longer latency, less planning; $p<.05$) as well as planning behavior and impulsivity measures (less consistency, greater impulsivity; $p<.05$). These associations were not observed for either inactivation condition. A negative association between choice latency and impulsivity was observed for the Epoch 2 condition only ($p<.05$). Together, these data indicate that mPFC is critical for either forming or holding a behavioral plan of action during intertemporal choice. Understanding the unique role that mPFC plays in promoting choices of delayed rewards provides a neurobiological target for treatment aimed at reducing impulsivity in clinical populations that are characterized by excessive impulsive choices.

Disclosures: S.M. White: None. **C.C. Lapish:** None. **C.L. Czachowski:** None.

Poster

241. Decision Making: Rodent Medial Prefrontal Cortex

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant AA023786

NIH P60-A007611

Title: Neural population activity in the rat medial prefrontal cortex underlying proactive behavior in a delay discounting task

Authors: *E. DE FALCO[1], M. MORNINGSTAR[1], S. M. WHITE[1], D. N. LINSENBARDT[3], C. C. LAPISH[2];
[2]Psychology, [1]IUPUI, Indianapolis, IN; [3]Addiction Neurosci. - Psychology, Indiana Univ. - Purdue Univ. Indianapolis, Indianapolis, IN

Abstract: Delay Discounting (DD) refers to the phenomenon by which a reward delivered in the future is valued less than one delivered immediately. DD tasks are widely used to asses impulsivity in a variety of experimental settings, however the neural computations underlying its decision-making process are poorly understood. Here, we used multi-electrode arrays to record the activity of neurons in the medial prefrontal cortex (mPFC) in awake, behaving Wistar rats performing a DD task. We used a within-session adjusting-amount DD procedure (Oberlin and Grahame, 2009), where the value of the immediate reward (I-value) was changed according to the animal's choice on the previous trial. This design allowed us to get an estimate of the subjective value of the delayed reward at the end of the session, and to compare trial choices based on their different prospective rewards (I-values). At the behavioral level, there were differences in the latency to choose both between immediate and delayed reward and between low and high I-value trials, with the longest latencies associated with immediate reward choices and low I-values. Accordingly, neural populations in mPFC were found to encode a variety of task dimensions necessary for the decision-making process, including I-value, reward, choice, and their overlap. In particular, using dimensionality reduction approaches we identified a subspace where neural trajectories in mPFC were clearly separated based on both choice and prospective value of the reward. The information content about the upcoming choice in mPFC spike trains (measured via mutual information) ramped gradually and peaked immediately prior to the choice. Interestingly, single trial neural activity on immediate vs delayed choices started to diverge immediately after the cue signaling the beginning of the trial was presented (around 10 seconds prior to the choice), which indicates encoding of the prospective action.In summary, these results characterize the broad role of mPFC neurons during decision-making in a DD tasks. Our observations are in agreement with previous behavioral findings (Linsenbardt et al., 2016), and reflect a mechanism of behavioral planning, where the upcoming choice is encoded in a prospective manner in the mPFC.

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Poster

241. Decision Making: Rodent Medial Prefrontal Cortex

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Topic: H.01. Animal Cognition and Behavior

Support: DFG JA 1999/3-1
ERC StG MEMCIRCUIT

Title: Neuronal repertoire for adaptive task-dependent decision making in mouse prefrontal cortex

Authors: *D. HÄHNKE, A. RANGANATH, T. W. BERNKLAU, S. N. JACOB;
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Abstract: The ability to modify behavior in the face of varying task demands is a central component of cognitive flexibility. In situations with insufficient evidence to initiate a goal-directed response, neural mechanisms must be implemented to refrain from premeditated actions and collect additional evidence. This requires an adaptive, task-dependent engagement of sensory and motor systems. To isolate a state of 'cognitive flexibility', we trained head-fixed mice on a two alternative forced-choice (2AFC) task in which animals indicated their responses by rotating a ball to the left or to the right using their forepaws. The ball's position could be continuously monitored as an index to the animals' 'state of mind'. Each trial comprised a sequence of two auditory stimuli. First, a context cue (100 ms) informed the animal if the subsequently required response direction was fully predictable (colored noise stimuli) or not (white noise). Following a delay period (500 ms), a response instruction signaled the required ball movement (leftward or rightward) to obtain reward. In trials with predictive cues, animals displayed a tendency to move the ball towards the cued direction during the delay period. Importantly, these trials were characterized by both better performance (4.5%, $p<0.0001$) and faster reaction times (21 ms, $p<0.0001$) compared to trials with non-predictive cues. These results suggest that the animals were able to use the predictive cues for motor preparation, while having to wait for additional sensory input in the unpredictive context. This behavioral pattern would likely involve the function of an executive brain region that tracks available evidence and adapts behavioral output on a trial-by-trial basis. While the animals performed the task, we extracellularly recorded the activity of a total of 2864 units (1540 single units, 1324 multi-units) in 61 sessions from the prelimbic region (PL) of the medial prefrontal cortex in 5 mice. 91% of PL units showed task-related activity. Subsets of units encoded the specific context and the response instruction (8% and 16% of all units, respectively). Notably, in the delay period following predictive cues, the PL population started to encode the upcoming response instruction. No such effect was seen in the unpredictive context. Together, these prefrontal

signals reflect the neuronal repertoire to implement the trained task and may aid downstream regions to initiate appropriate behavioral responses. Our findings warrant exploration of the network comprising the prefrontal cortex and its linked regions to provide an in-depth description of the chain of events associated with cognitive flexibility.

Disclosures: D. Hähnke: None. **A. Ranganath:** None. **T.W. Bernklau:** None. **S.N. Jacob:** None.

Poster

241. Decision Making: Rodent Medial Prefrontal Cortex

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Topic: H.01. Animal Cognition and Behavior

Support: Yale Kavli Foundation

Title: The causal contributions of medial prefrontal cortex to value-based decisions in mice

Authors: *H. ATILGAN, C. MURPHY, J. A. FRAGA, A. KWAN;
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Abstract: Learning from experience is essential to the optimization of behavior. In particular, we learn from past choices and outcomes to infer the predicted values of the actions to be taken. Then based on the values, we may select an informed choice. However, despite the many neural correlates identified, we still do not have a clear picture for how values are computed and translated into informed behavior. Here, we trained head-fixed mice to perform a two-armed bandit task. Animals based their decisions on past choices and reinforcements, consistent with having an internal representation of action values. To determine the causal contributions of the medial prefrontal cortex, we tested the animals before and after an excitotoxic lesion of the medial secondary motor cortex (mM2). We found that a unilateral mM2 lesion led to side-specific effects on the animal's ability to learn from past choices. To quantify the decision-making process, we fitted the animal's choice behavior with Q-learning models to extract learning parameters such as learning rate, forgetting rate, and inverse temperature. Altogether, the results provide insights into the causal involvement of mouse mM2 in value-based decision making.

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Poster

241. Decision Making: Rodent Medial Prefrontal Cortex

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Program #/Poster #: 241.08/W10

Topic: H.01. Animal Cognition and Behavior

Support: Wellcome Trust Grant 209558
Simons Foundation Award 543029

Title: Visual decision-making in a dynamically changing environment: Comparing mouse behavior to ideal observer models

Authors: L. ACERBI[1], A. E. URAI[2], V. AGUILLON-RODRIGUEZ[2], D. E. ANGELAKI[3], N. BONACCHI[4], M. CARANDINI[5], F. CAZETTES[4], G. A. CHAPUIS[5], A. K. CHURCHLAND[2], Y. DAN[6], E. E. J. DEWITT[4], M. FAULKNER[5], M. HAUSSER[5], F. HUI[6], I. C. LARANJEIRA[4], Z. F. MAINEN[4], G. T. MEIJER[4], N. J. MISKA[4], T. D. MRSIC-FLOGEL[5], J.-P. NOEL[3], A. PAN-VAZQUEZ[7], C. ROSSANT[5], K. Z. SOCHA[5], M. J. WELLS[5], C. J. WILSON[3], O. WINTER[4], I. B. WITTEN[7], A. M. ZADOR[2], .. IBL COLLABORATION[5], *A. POUGET[1];
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Abstract: How accurately do animals track the statistics of a dynamic decision-making environment? We model here behavioral data from a large cohort of mice (N=24) performing a visually guided decision-making task across multiple laboratories, all part of the International Brain Laboratory (IBL).
On each trial, mice saw a Gabor patch of varying contrast on the left or right side of a screen, and reported the stimulus location by turning a wheel in order to obtain a liquid reward upon correct response (Burgess et al., 2017). Mice performed sessions in which the probability of a "Left" stimulus changed between blocks of trials. Each session started with a single "unbiased" 90-trial block with P(Left) = 0.5, followed by blocks that alternated between P(Left) = 0.2 and P(Left) = 0.8. Each block length was drawn randomly from an exponential distribution (time constant 60 trials), truncated between [20,100] trials.
We investigate whether and how mice use dynamically updating beliefs about these changing blocks in their decision process. To do so, we fitted three different models to the behavioral data. The "fixed" model ignores the block structure and assumes a fixed estimate of P(Left) at all times. The "exact" model assumes that mice know the exact change-point parameters, including the set of block probabilities and the distribution of block lengths, and iteratively update the posterior probability over the time since the last change-point (Norton et al., 2018). Finally, the

"flexible" change-point model is similar to the exact model except that the change-point parameters are allowed to differ from the true task parameters. We fitted the models to data from each mouse (~15 sessions and ~12000 trials per mouse).
Bayesian model comparison via BIC overwhelmingly favored the "flexible" change-point model. In particular, the inferred task parameters differed substantially from the true parameters (e.g., time constant of change-point ~10 trials instead of 60, block probabilities ~0.5 and 0.6 instead of 0.2 and 0.8). These results show that mice dynamically adjust their behavior following the probability switch across blocks but do not fully learn the statistics of the task.

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Poster

241. Decision Making: Rodent Medial Prefrontal Cortex

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Program #/Poster #: 241.09/W11

Topic: H.01. Animal Cognition and Behavior

Support: ERC Grant PRIORS-683209

Title: Decoupled evidence accumulation and reaction times in expectation-guided perceptual decisions

Authors: *L. HERNÁNDEZ-NAVARRO[1], A. HERMOSO-MENDIZABAL[1], D. DUQUE DONCOS[1], J. DE LA ROCHA[1], A. HYAFIL[2];
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Abstract: In perceptual categorization tasks, reaction times (RTs) and choices do not only depend on current sensory evidence, but also on subject's urgency and prior expectations. To study these dependencies, we trained rats in a reaction time auditory discrimination task. Standard decision-making Drift Diffusion Models (DDMs) predict a relation between mean RTs and evidence strength. However, rats' express responses (RT&lt80ms, ~35% of trials) showed stimulus-independent RTs. Choices in such express responses, on the other hand, did depend on the stimulus (and increasingly so with longer RTs). Moreover, in ~20% of the trials rats responded shortly before the offset of the fixation period and before the stimulus was presented. To account for these observations, we propose the Dual DDM, a novel model in which rats'

responses are triggered by either an urgency signal or by sensory evidence accumulation, which are independently represented by two distinct stochastic integrators. The response is triggered whenever any integrator reaches threshold. The urgency integrator is initiated in anticipation of the stimulus onset, whereas the latter starts with stimulus onset after some sensory delay. The choice of the rat is always set by the accumulated evidence at response time. Because rat responses are largely influenced by trial history, we studied the impact of expectations on the urgency and evidence integrators. We introduced correlations in the stimulus sequence to induce trial-dependent expectations towards repeating or alternating the previous response. We first found that, surprisingly, post-error slowing arose from the combination of a lower stimulus sensitivity in the evidence integrator and a slowing of the urgency integrator. Second, the lateral bias (accumulated win-stay side bias) arises as a constant bias in the drift of the evidence integration, while the transition bias (accumulated bias to repeat or alternate the previous response) is implemented as an initial offset. Finally, the urgency integrator is also affected by expectations, although in a different manner: it speeds up under growing bias to alternate but, surprisingly, it slows down under increasing bias to repeat. In conclusion, standard models of decision making predict a direct relation between accumulated evidence and RTs, which is inconsistent with experimental observations in rats. A novel dual model, grounded on an independent integration of urgency and evidence, could capture rat behavior, and decouple the impact of distinct history biases on RTs and choices.

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Poster

241. Decision Making: Rodent Medial Prefrontal Cortex

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Support: William and Ella Owens Medical Research Foundation
Veterans Affairs I01BX003512

Title: Infralimbic BDNF is necessary for the therapeutic effects of extinction after chronic stress in male and female rats

Authors: *D. PAREDES, D. A. MORILAK;
Univ. of Texas Hlth. Sci. Ctr. San Anto, San Antonio, TX

Abstract: Stress-related psychiatric disorders share a common dysfunction in the medial prefrontal cortex (mPFC), such that individuals with these disorders perform poorly on mPFC-dependent behaviors such as set-shifting, a form of cognitive flexibility. We can model stress-

induced cognitive dysfunction in set shifting using chronic unpredictable stress (CUS) in rodents and measuring their performance on the attentional set shifting test (AST). Behavioral therapies such as exposure therapy can be effective in ameliorating cognitive dysfunction associated with PTSD and depression. However, little is understood about the neurobiological mechanisms underlying behavioral interventions. We have previously shown that fear extinction, (i.e. learning that an innocuous cue previously associated with a fearful stimulus no longer predicts that stimulus) can be used to model the effects of exposure therapy on cognitive flexibility that has been compromised in chronically stressed rats. Further, we have shown that fear extinction (FE) requires *de novo* protein synthesis in the infralimbic (IL) region to exert its therapeutic effects on set shifting. Additionally, extinction learning induces the phosphorylation of the BDNF receptor TrkB at the Y515 site, associated with activation of the PI3K/Akt pathway. Thus, we hypothesized that: 1) infralimbic BDNF may mediate the protein synthesis and plasticity processes that are necessary for the therapeutic effects of extinction in stressed animals, and 2) extinction learning requires the Akt pathway to exert these effects. To test these hypotheses, male and female rats were chronically implanted with guide cannulae targeting the IL. Rats underwent chronic stress or control treatment, and prior to extinction, received either a neutralizing antibody against BDNF or a sheep IgG control into the IL. In a separate experiment, animals received a microinjection of either vehicle (0.5% DMSO) or an Akt inhibitor directly into the IL prior to extinction learning. 24 hr after extinction, animals were tested on the AST to evaluate set shifting performance. Blocking BDNF at the time of extinction blocked the therapeutic effect of extinction on set shifting after stress in both sexes. Thus, infralimbic BDNF is necessary for the therapeutic effects of extinction on set shifting after chronic stress in both sexes. Further, preliminary results suggest a role for Akt signaling in the effects of extinction on set shifting after stress. These experiments identify key molecular processes that initiate plasticity mechanisms during extinction learning that correct stress-induced dysfunction in the infralimbic cortex.

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Poster

241. Decision Making: Rodent Medial Prefrontal Cortex

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Topic: H.01. Animal Cognition and Behavior

Support: Simons Foundation SCGB AWD543027
Wellcome Trust 209558

Title: State-dependent modeling of psychophysical behavior during decision making

Authors: *Z. C. ASHWOOD[1], N. A. ROY[1], A. E. URAI[2], V. AGUILLON RODRIGUEZ[2], N. BONACCHI[3], F. CAZETTES[3], G. A. CHAPUIS[4], A. K. CHURCHLAND[2], M. FAULKNER[4], F. HU[5], C. KRASNIAK[2], I. C. LARANJEIRA[3], G. T. MEIJER[3], N. J. MISKA[4], J.-P. NOEL[6], A. PAN-VAZQUEZ[1], C. ROSSANT[4], K. Z. SOCHA[4], I. R. STONE[1], M. J. WELLS[4], C. J. WILSON[6], O. WINTER[3], .. IBL COLLABORATION[4], J. W. PILLOW[1];
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Abstract: Animals performing perceptual decision-making tasks often exhibit "lapses" — choices that are independent of the sensory evidence, which can produce errors even on trials with strong evidence [Wichmann and Hill, 2001; Busse et al., 2011]. Lapses are surprisingly frequent in some animals, and failure to account for them can result in inaccurate estimates of sensitivity and psychometric function parameters [Wichmann and Hill, 2001]. One approach for modeling lapses consists of a mixture model, where an independent coin flip on each trial determines whether the animal uses task-relevant variables to make a decision or lapses. However, this model is limited by two assumptions: (1) that lapses arise independently across trials, and are thus uncorrelated within a session; and (2) that lapses are purely noise-driven events that cannot be predicted from experimental covariates.
Here we describe a discrete state-space model for decision-making behavior that overcomes these limitations. Our model is a Hidden Markov Model (HMM) with multinomial Generalized Linear Models (GLMs) describing both decisions and transitions between states, which we refer to as the GLM-HMM. The model has K discrete internal states, each of which corresponds to a different mode of decision-making behavior. Transitions between these states are parametrized by GLMs that allow for learned functions of the task covariates (sensory evidence as well as well as task-irrelevant variables like reward, stimulus history, or elapsed time within a session) to affect the probability of the next state. Decision-making is also governed by state-specific GLMs, which describe the mapping from task covariates to the animal's choice on each trial. This model can incorporate traditional lapses with a state where the GLM weights on the sensory stimulus are zero; however, the GLM-HMM provides a richer framework for lapse-related behavior by modeling (1) multiple states with different decision-making strategies, and (2) the factors governing transitions between these states.
We use the GLM-HMM to analyze mouse behavior in a visually guided decision-making task, using data collected by the International Brain Laboratory. The task required mice to turn a wheel to report whether a grating appeared on the left or right side of a screen [Burgess et al., 2017]. We examine the number of states required to characterize the sensory decision-making behavior of mice performing this task, and the task covariates governing decisions in each state. We compare these results to fits with alternative lapse models in order to understand the strategies underlying decision-making behavior and the dependencies between them.

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Poster

241. Decision Making: Rodent Medial Prefrontal Cortex

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Alfred P. Sloan Foundation
Pew Foundation
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Title: Alpha and gamma power in medial prefrontal cortex reinforces reward contingencies and predicts asymptotic performance in a cognitive flexibility task

Authors: *H. J. GRITTON, N. M. JAMES, J. C. NOCON, M. ABDULKERIM, K. K. SEN, X. HAN;
Biomed. Engin., Boston Univ., Boston, MA

Abstract: The ability to adapt to ever-changing environments represents a key tenet of cognitive function and executive control. Fundamental to this process is the integration of sensory cues, decision making and the executive control of behavior. The processes by which cortical circuits are modified by changes in task rules are not completely understood, but could involve changes in prefrontal regions that are ultimately conveyed to sensory regions important for stimulus monitoring. To understand the functional role of cognitive flexibility, and how changes in task rules influence frontal and sensory regions, we recorded from auditory (AC) and medial prefrontal cortex (mPFC) while mice performed an auditory extinction learning task. Prior to recording, animals were trained to lick for a small water reward that had been paired with two distinct tones separated by 1.5 octaves. Once the associations were well learned, recordings were made from animals when the reward was withdrawn from one of the two tones while the other was maintained. We found the change in task rules produced immediate and robust changes in both higher gamma (50-75 Hz) and alpha (8-12 Hz) power during a confusion interval that occurred prior to animals learning to modify their behavior. The changes in gamma and alpha were unique to different trial types and their augmentation differed in relationship to the reward window. Gamma was enhanced during reward intervals only for still rewarded tones. In contrast, alpha power rose at the conclusion of the reward window and only following the extinguished tone. This increase in power was observed in mPFC before AC, suggesting that mPFC is recruited earlier in extinction learning than AC. Furthermore, the strength of alpha power in the

mPFC was also strongly predictive of the asymptotic performance the animal would later achieve in the training session. We found that both changes in gamma and alpha power persisted even as performance improved, suggesting these rhythms may have an ongoing role in the maintenance of newly learned associations, or represent components of expected value under the new outcome contingencies.

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Poster

241. Decision Making: Rodent Medial Prefrontal Cortex

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Topic: H.01. Animal Cognition and Behavior

Support: NSF 1250104

Title: Patch-based foraging by head-fixed mice in virtual reality

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Abstract: Animals and humans adjust their response to a sensory stimulus to its learned meaning. For example, when a sound is associated with reward, animals learn to respond with an appropriate action to harvest the reward. The neural substrates of these ‘value-based’ decisions have been extensively studied in neuroscience, primarily using behaviors that temporally discretize the decision process. For example, in classic two-alternative forced-choice (2AFC) or Go/No-go paradigms, sequences of time windows contain e.g.: a contextual cue, a sensory stimulus set, a reward or other feedback, and interspersed delays. This sequence is repeated in a similarly rigid sequence of trials. Such behaviors are ideal for temporally isolating decision process components, and thus have greatly informed underlying brain mechanisms. However, these tasks differ dramatically from the natural decision-making contexts in which the brain evolved. Behavioral ecologists have found that natural value-based decisions often occur in patch-based foraging contexts, with 3 defining features: 1) resources are encountered one at time (e.g. food, water, or a mate); 2) resources deplete with usage (e.g. nuts in a tree run out); and 3) upon depletion, animals incur the ‘cost’ of traveling to the next resource (patch). Thus, animals make an ongoing decision, balancing the marginal return in a patch with the cost of traveling to the next patch, using a learned internal representation of the environment. Well-developed theory, such as the marginal value theorem, has been developed and supported by numerous

studies in behavioral ecology. Yet patch-based foraging has rarely been studied in neuroscience. We developed a patch-based foraging task for head-fixed mice on a cylindrical treadmill in a virtual environment defined by auditory cues. A tone cloud indicates that mice can lick a spout for sucrose solution. The reward volume decreases, or time between rewards increases, with time in patch. The mouse may leave a patch at any time by running, indicated by pink noise. They enter the next patch (indicated by tone cloud) after traversing a fixed distance on the treadmill. Analyses of walking and licking indicate that mice learn to harvest reward in patches, minimize travel time between patches, and adjust in-patch lick rates to match the depletion rate. In recordings from dorsal anterior cingulate cortex (dACC) with silicon probes (Masmanidis lab, 128-channel) during behavior (N=2), many units have altered firing rates prior to patch leaving, consistent with previous findings in monkeys (Hayden et al., 2011). We are investigating the computational roles of dACC and other areas in patch-based foraging decisions.

Disclosures: J. Webb: None. **A. Banta:** None. **Z.H. Mridha:** None. **W. Zhang:** None. **C. Kemere:** None. **M.J. McGinley:** None. **D. Lee:** None.

Poster

241. Decision Making: Rodent Medial Prefrontal Cortex

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 241.14/W16

Topic: H.01. Animal Cognition and Behavior

Support: Marsden Grant UOO1617

Title: A novel weight lifting task to investigate persistence in rodents

Authors: *B. S. PORTER, K. HILLMAN;
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Abstract: One function of the mammalian anterior cingulate cortex (ACC) may be to motivate behavior when an animal is presented with a difficult but valuable behavior to pursue. Rats with lesions to or inactivation of the ACC will forgo expending effort (barrier climbing, cognitive effort) for a high reward and opt instead to expend little effort for a small reward. ACC single units have been shown to fire preferentially to the most valuable, effort-discounted option available. The effort-discounted value computation carried out by the ACC may serve as a top-down control signal to motivate behavior towards difficult but “worth-it” choices. However, to date rodent tasks that probe persistence in the face of difficulty have drawbacks as well as mixed results as to the role the ACC plays. Lever pressing tasks using fixed or progressive ratios may cause rats to stop persisting due to satiation or boredom rather than effort challenges. In contrast, effort based choice tasks can be difficult to interpret as rats may switch their choice from high to low effort but continue to persist at the task overall. Furthermore, when effort is tested with

barrier climbing, ACC lesions result in rats opting for low effort options. However, when effortful decision making is tested with weighted lever pressing, ACC lesions have no effect on behavior. In order to elucidate the role of the ACC in persistence we developed a novel, non-choice rodent weight lifting task. The task requires rats to pull a rope attached to a variable weight to get a fixed reward. The weight could be manipulated within session to increase effort. Adult male Sprague Dawley rats performed the task well and could achieve weights upwards of 40% of their body weight. Individual rats had distinct levels of persistence at the task. Infusions of GABA agonists Baclofen and Muscimol into the ACC did not influence task performance. These data help to elucidate the role of the ACC in effortful behaviors and show further support that not all types of effort are processed equally in the ACC. Overall, novel weight lifting task provides a new behavioral paradigm for testing effort and persistence in rodents.

Disclosures: B.S. Porter: None. **K. Hillman:** None.

Poster

241. Decision Making: Rodent Medial Prefrontal Cortex

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 241.15/W17

Topic: H.01. Animal Cognition and Behavior

Support: Marsden Fund UOO1212

Title: Dysfunction in the anterior cingulate cortex and the ventral tegmental area in relation to decision making and motivation in an animal model of schizophrenia

Authors: *E. R. CROY[1], T. W. ELSTON[2], D. K. BILKEY[1];
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Abstract: Schizophrenia is associated with deficits in decision making and learning. The anterior cingulate cortex (ACC) and the ventral tegmental area (VTA) have been linked to decision making and motivation respectively, and dysfunction in these regions has been linked to schizophrenia. Here we examined VTA and ACC activity in a maternal immune activation (MIA) rodent model of schizophrenia risk. A cost-benefit reversal task was performed by 22 adult male rats (10 MIA) whilst local field potentials (LFPs) were recorded from their ACC and VTA. The cost-benefit task was a T-maze with one arm offering a higher cost (barrier; HC) and higher reward (HR); the other arm had a lower cost (no barrier; LC) and a lower reward (LR). Halfway through a recording session the cost and benefit location reversed. Control rats chose the LCLR choice, which corresponded to the previous session's final HCHR choice, significantly more often than MIA rats (and chance) on the first trial of each session. Control rats also showed a decrease in HCHR choices as they approached the reversal, but an increase as they approached

the end of the session. This contrasted to MIA rats which displayed little change in percentage of HCHR choices approaching the reversal and at the end of the session. Post-reversal MIA rats had significantly lower HCHR choices than controls, however both groups showed an increase in HCHR choices during post-reversal. MIA and control rats also demonstrated different levels of low-beta and delta LFP activation in the VTA and the ACC in the decision-making part of the maze. When equated across behaviour, MIA rats had a relatively consistent level of ACC/VTA power across all choice options while controls showed a dip in power on trials were choices were around 50% LCLR/HCHR. These results suggest that the MIA rats are failing to effectively incorporate previous knowledge into their decision-making. They perform at chance at the start of the session and don't appear to anticipate the upcoming reversal. They also demonstrate inflexibility, during reversal learning. These behaviours suggest a failure in longer-term memory, and a greater reliance on recent outcomes. This suggests that they focus on the more immediate timeframe, diminishing the importance of information gathered during previous sessions. Schizophrenia is associated with similar difficulties in cognitive flexibility, perseveration with a default option, and a failure to use new knowledge to adapt behaviour. The LFP data suggest that the ACC and VTA may be involved in this deficit, failing to discriminate between HCHR and LCLR choices in MIA rats, while signalling exploratory LCLR, and HCHR choices in the controls.

Disclosures: E.R. Croy: None. **T.W. Elston:** None. **D.K. Bilkey:** None.

Poster

241. Decision Making: Rodent Medial Prefrontal Cortex

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant P01-HD080679

Title: Role of medial prefrontal cortex in rat category learning

Authors: *M. B. BROSCHARD, J. KIM, J. H. FREEMAN;
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Abstract: The process of category learning involves determining which physical features are typical of the category (e.g., category-relevant features) and which features are less exclusive to that category (e.g., category-irrelevant features). Attention should be orientated towards the category-relevant features accordingly. Multiple theories of human category learning, for instance COVIS (Competition between Verbal and Implicit Systems) and EpCon (Episodes-to-Concepts), implicate the prefrontal cortex in allocating attention towards category-relevant information. In the current study, we tested this prediction by administering lesions in the medial

prefrontal cortex (mPFC) of rats. Then, using a touchscreen apparatus, rats were trained to categorize circular stimuli with black and white gratings that changed in spatial frequency and orientation. Some tasks required attention to one stimulus dimension (rule-based; RB tasks), whereas other tasks required attention to both stimulus dimensions (information integration; II tasks). After 15 training sessions, we examined category generalization by presenting testing sessions containing novel exemplars. We found that compared to sham controls, rats with mPFC lesions were impaired on RB, but not II, tasks. Additionally, fitting the Generalized Context Model to the generalization data revealed that the mPFC lesions impaired selective attention for rats learning the RB tasks. Together, these results support models of category learning that posit a role for the PFC in directing attention towards category-relevant information. Future research will examine how mPFC directs attention.

Disclosures: **M.B. Broschard:** None. **J. Kim:** None. **J.H. Freeman:** None.

Poster

241. Decision Making: Rodent Medial Prefrontal Cortex

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Title: Prefrontal cortex enables cognitive flexibility and contextual bias

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Abstract: Individuals constantly make inferences about the environment in which they operate. Processing and interpreting the stream of multisensory information in the surroundings enables adjustable decision-making, optimized actions and consequently, maximization of reward. This process underlies the foundation of cognitive flexibility and is essential for resilient and adaptive behavior. It has been proposed that the prefrontal cortex (PFC) is a crucial hub controlling this function. However, the precise role of this brain area for computing such process remains elusive.
We developed a modified version of a self-initiated two-alternative forced choice task in head restrained mice that learned to associate clouds of tones with different degrees of ambiguity in predicting reward in the left vs. right side. Mice had furthermore learned to flexibly adjust their

behavioral strategies depending on two different multisensory stimuli (hereafter referred to context A and B) preceding the tone clouds and presented randomly. Context A predicted the same amount of reward on both sides while context B predicted a high reward volume on one side and no reward on the other. We observed that mice ignored this contextual information to process sensory selection of unambiguous tone cloud. By contrast, under conditions of sensory ambiguity, mice choices were biased toward the highest rewarded side in context B but not in context A.
To test the role of medial PFC (mPFC) in processing such context-dependent bias, we optogenetically silenced mPFC neurons during the contextual epoch using ArchT. This manipulation suppressed the bias, suggesting that mPFC is critical for controlling this behavior. Using calcium imaging while mice were performing the task, we further confirmed the involvement of mPFC since ensembles of neurons encoded contextual cues. More importantly, we found that a subpopulation of mPFC neurons specifically encoded the context-dependent bias, suggesting that they don't merely respond to sensory cues but also regulate the biased strategy of the mouse.
Taken together, those results suggest that mPFC enables context-dependent behavioral bias, a cognitive flexibility important under situations of sensory ambiguity.

Disclosures: O. Gschwend: None. **B. Li:** None.

Poster

241. Decision Making: Rodent Medial Prefrontal Cortex

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant P20 GM113109-01A1

Title: Prelimbic cortex inactivation during training does not impair later devaluation in a cued-trial multiple-response/multiple reinforcer operant devaluation task in rats

Authors: *H. FISHER, A. PAJSER, C. L. PICKENS;
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Abstract: Devaluation is a task often used to model flexible goal-directed action, the ability to adaptively modify behavior when the value of a reinforcer changes. In an experiment with pre-training inactivations, we previously found that basolateral amygdala (BLA) and mediodorsal thalamus (MD), but not orbitofrontal cortex (OFC), were necessary to learn the initial information needed to guide later devaluation. Due to the parameters of our cued-trial multiple-response/multiple-reinforcer operant devaluation task, rats could either use stimulus-outcome (S-O) associations using the unique cuelights above each lever or response-outcome (R-O)

associations using the spatial lever location to maintain goal-directed action. Here, we tested male Long Evans rats (n=24) in a cue-switching experiment to determine the strategy rats use to complete our task (attending to the discrete light cue or spatial lever location). In the Cue Normal group, the rats received the same lever-light compound configurations in the devaluation test as during training. In the Cue Switched group, the cuelights above the levers were switched during the devaluation tests compared to their position in training. Both groups exhibited a devaluation effect based on the lever location, suggesting rats without neurobiological manipulations rely on the spatial lever location to guide behavior in our devaluation task. Because rats primarily use an R-O strategy, putatively mediated by the prelimbic cortex (PL), we then sought to determine whether PL was required for learning the initial information required for future goal-directed action in our task. In male and female Long Evans rats (n=68), we inactivated PL during initial training and found that both the PL inactivation and control groups showed a devaluation effect. The lack of OFC and PL involvement in our task, despite rats naturally preferring a presumably PL-mediated strategy, suggests that rats can compensate for the loss of PL function by using S-O associations, presumably supported by OFC, to guide goal-directed action in our task. Future studies will verify that OFC and PL can compensate for each other to maintain intact goal-directed action in our task when the functioning of one of them is impaired.

Disclosures: H. Fisher: None. **A. Pajser:** None. **C.L. Pickens:** None.

Poster

241. Decision Making: Rodent Medial Prefrontal Cortex

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 241.19/W21

Topic: H.01. Animal Cognition and Behavior

Support: Swiss National Science Foundation (SNSF)

Title: Contribution of primary somatosensory and medial prefrontal cortices to sensorimotor transformation during goal-directed behavior in mice

Authors: *A. ORYSHCHUK, V. ESMAEILI, E. CHARRIÈRE, C. C. H. PETERSEN, S. CROCHET;
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Abstract: A fundamental goal of neuroscience is to understand how sensory stimuli are represented in the brain and how this information guides behavior. Studies in non-human primates and rodents, performing sensory detection or discrimination tasks, have shown a gradual transformation of the sensory signals into decision/motor signals from sensory to frontal areas. Primary sensory areas encode mostly the physical features of the stimulus, whereas the response of frontal areas (e.g. the prefrontal cortex), co-varies with the decision as well as the

stimulus strength. In this study, we investigated the role of primary whisker somatosensory cortex (wS1) and medial prefrontal cortex (mPFC) in sensorimotor transformation in mice during goal-directed behavior. We used high-density silicon probes to record simultaneously the neuronal activity in wS1 and mPFC while mice performed a psychophysical whisker-based detection task. Mice were trained to report a brief whisker deflection of variable amplitude by licking a reward spout. Neurons in wS1 responded to the whisker stimulus with a typical biphasic response, including an early (before 50 ms) and sharp increase in firing rate followed by a late (50-300 ms) secondary response. Reducing the evoked neuronal activity in wS1 using mild optogenetic inactivation caused a rightward shift of the psychometric function with a significant decrease in performance for near-threshold stimuli, consistent with the hypothesis that evoked activity in wS1 is causally involved in the perception of the sensory stimulus. Neurons in mPFC exhibited a longer latency response to the whisker stimulus, that peaked around 50 ms and lasted a few hundreds of ms. The neuronal response in both wS1 and mPFC increased monotonically with the amplitude of the whisker stimulus. Comparing trials with different behavioral outcomes (i.e. Hit vs Miss) revealed interesting differences between the two regions. wS1 neurons' early response encoded the stimulus in both trial types while mPFC neurons' activity correlated with the stimulus amplitude only in Hit but not Miss trials. Recordings during the first training session showed that a whisker-evoked response in mPFC was observed only in mice that learned the task while wS1 neurons encoded the whisker stimulus regardless of learning. Together, our preliminary results suggest a biased, but mixed, encoding of sensory- and decision-related information in wS1 and mPFC.

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Poster

241. Decision Making: Rodent Medial Prefrontal Cortex

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 241.20/W22

Topic: H.01. Animal Cognition and Behavior

Title: Tracking choice and reward value in single neurons over several days

Authors: *M. MOLTESEN, J. S. L. YEPEZ, D. KVITSIANI;
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Abstract: In electrophysiological recordings it is difficult to track activity of single neurons over several days. This is due to the stability of chronically implanted electrodes and/or spike sorting algorithms that make it difficult to identify the same neurons over multiple recording sessions. Here we report the method that allows us to follow the same single units over days and weeks in behaving mice. To achieve this goal we used template based spike sorting algorithm Dsort. Dsort

uses unique spatial-temporal spike waveforms to identify spikes from multiple recording sessions. With this tool we have analyzed how single neurons represent decision variables over days in a probabilistic reward foraging task. The behavioral task uses trial-based version of variable interval schedules of reinforcements that naturally balances perseverance and alternation of choices. Using this behavioral paradigm, we evaluate if single neurons keep representation of reward and choice history over days. We are in the process of analyzing the data.

Disclosures: M. Moltesen: None. **D. Kvitsiani:** None. **J.S.L. Yepez:** None.

Poster

241. Decision Making: Rodent Medial Prefrontal Cortex

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Program #/Poster #: 241.21/W23

Topic: H.01. Animal Cognition and Behavior

Support: PICT 2016-2145
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Title: Inhibition of neuronal activity in the prefrontal cortex after systemic administration of fluoxetine during the execution of an operant conditioning task

Authors: A. E. PEREYRA[1], C. J. MININNI[1,2], *B. S. ZANUTTO[1,2];
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Abstract: The prefrontal cortex (PFC) is a main brain region controlling high-level executive functions and goal-directed behaviors. Several neuropsychiatric disorders are related to the deficits in cognitive and emotional processes subserved by PFC. Serotonergic system plays an important role in regulating prefrontal function. It is also involved in depressive disorders, and fluoxetine, a selective serotonin reuptake inhibitor (SSRI), has been a successful antidepressant drug. Although it is well known the effect of fluoxetine on the concentrations of monoamines in the cortex, such as 5-HT, dopamine and noradrenaline, and in other regions related to motivated behaviors, its effect on the activity of the PFC neurons *in vivo* is not well understood, especially during the execution of a behavioral conditioning task. In this study we performed extracellular recordings of PFC neurons of head-fixed Long Evans rats, during the execution of an operant conditioning task. Rats had to make a lick action after an auditory cue, in order to obtain a drop of water as a reward. Recordings started after behavioral performance was stable and above 80% of correct responses. After 10 sessions of control recordings, fluoxetine was administered on a daily basis (10 mg / kg / day, oral dose) and another 10 sessions were performed. We observed a progressive reduction in the PFC mean firing rate (FR) along sessions after fluoxetine treatment

onset, and an increment in mean FR occurs during the consumption for control and fluoxetine recordings. Interestingly, a reduction in FR occurs immediately before cue onset during fluoxetine sessions, which is absent in control recordings. Moreover, the mean FR and the mean correlation between pairs of neurons during consumption's time were significantly lower for fluoxetine recordings. Our results are consistent with an inhibitory effect of chronic administration of fluoxetine on the pyramidal neurons of the PFC due to a higher excitability of GABAergic interneurons, and contribute to the understanding of the effect of SSRIs on neuronal activity in PFC during the execution of a cognitive task.

Disclosures: A.E. Pereyra: None. **C.J. Mininni:** None. **B.S. Zanutto:** None.

Poster

242. Learning and Memory: Cortical-Hippocampal Interactions I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 242.01/W24

Topic: H.01. Animal Cognition and Behavior

Support: MOST 106-2320-B-006-026-MY3

Title: Distinct role of the agranular and granular retrosplenial cortex in remote contextual fear memory in mice

Authors: *T.-C. TSAI[1], K.-S. HSU[1,2];
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Abstract: The retrosplenial cortex (RSC) is a dorsomedial parietal area involved in the acquisition, consolidation and retrieval of contextual fear memory. The RSC receives diverse inputs from dorsal hippocampal and parahippocampal areas and sends axons to various cortical areas. Anatomically, the RSC can be divided into dorsal agranular (RSA) and ventral granular (RSG) parts; however, their individual role in the retrieval of remote memory remains unclear. Here, combining viral circuit tracing with chemogenetic approaches, we identified the RSA and RSG microcircuits and their role in the retrieval of remote contextual fear memories. We found that the RSC has strong reciprocal connections with the hippocampus and postrhinal cortex (POR). Axons from the RSA formed connections with the lateral entorhinal cortex (LEnt) and POR, and the RSG received a direct input from the hippocampal dentate gyrus (DG). In addition to these connections, synaptic connection was made between the RSA and RSG. In addition, retrieval of remote contextual fear memories activates neurons in the RSA, RSG, POR, LEnt, amygdala and DG. Pharmacological blockade and chemogenetic silencing of the RSG neurons impaired the retrieval of remote memory. Collectively, these data are the first demonstration that the RSG is specifically involved in the retrieval of remote contextual fear memory.

Disclosures: **T. Tsai:** None. **K. Hsu:** None.

Poster

242. Learning and Memory: Cortical-Hippocampal Interactions I

Location: Hall A

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Title: Differences in intrinsic connectivity patterns of layer Vb neurons between the lateral and medial entorhinal cortex

Authors: *S. OHARA[1,2], R. R. NAIR[1], S. BLANKVOORT[1], C. KENTROS[1], M. P. WITTER[1]; [1]Kavli Inst. for Systems Neurosci. and Ctr. for Neural Computation, NTNU, Trondheim, Norway; [2]Lab. of Systems Neurosci., Tohoku Univ. Grad. Sch. of Life Sci., Sendai, Japan

Abstract: The entorhinal cortex (EC), which is the major gateway between the hippocampus and the neocortex, is composed of functionally distinct subdivision, lateral and medial EC (LEC and MEC). LEC processes information about odors and objects, whereas a large proportion of neurons in MEC code spatial information. One factor which may contribute to these functional differences is the unique local circuit of these two regions. Here, we focused on the local circuits constituted by layer V neurons in deep-sublayer Vb (LVb). LVb neurons are main recipients of hippocampal projections, and project intrinsically, mediating two circuits in the hippocampus-memory system: 1) a feedback projection, sending information back to the hippocampus via neurons in layer II/III (LII/III), and a hippocampal output circuit to telencephalic areas by projecting to the superficial-sublayer a of layer V (LVa) (Ohara et al., 2018). To examine the differences of these local circuits between MEC and LEC, we used a transgenic mouse which expresses tetracycline-controlled transactivator (tTA) mainly in EC LVb excitatory neurons (MEC-13-53D, Blankvoort et al., 2018). The channelrhodopsin variant (oChIEF) and a fluorescent protein (citrine) were specifically expressed in LVb neurons with tTA dependent virus, AAV2/1-TRE-Tight-oChIEF-citrine, and the axonal distribution of LEC LVb neurons and MEC LVb neurons were assessed. We also examined the differential projection of LVb neurons to LII/III/Va neurons by performing whole-cell patch-clamp recordings from cells in these layers while optically stimulating LVb fibers in acute slices. The projections from LVb-to-LII/III were similar in LEC and MEC, and GFP-labeled axons were more numerous in LIII than in LII. In line with this anatomical observation, LIII neuronal excitatory responses were larger than those

in LII in both regions. This indicates that the EC-hippocampal loop favors the LIII-to-CA1/subicular projection rather than the LII-to-DG/CA3 one in both regions. In contrast, the LVb-to-LVa projection differed between LEC and MEC. In LEC the LVb axons were densely distributed in LVa, whereas in MEC they avoided LVa. The excitatory responses in LVa neurons after LVb stimulation were significantly lower than in LIII neurons in MEC but not in LEC. This result indicates that the hippocampal output circuit is more prominent in LEC than in MEC. These findings may provide new insights to unravel the neuronal basis underlying the phenotypical differences between LEC and MEC, and also indicate that these two regions may contribute differently to memory consolidation.

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Poster

242. Learning and Memory: Cortical-Hippocampal Interactions I

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Title: Encoding temporal traces within hippocampal-cortical circuits

Authors: *A. CICVARIC[1], Y.-Z. WANG[2], N. YAMAWAKI[3], L. Y. REN[8], M. MEYER[8], V. JOVASEVIC[1], A. L. GUEDEA[4], Y. HAN[9], O. A. MORENO-RAMOS[1], N. KHALATYAN[5], V. GRAYSON[10], G. M. SHEPHERD[6], J. N. SAVAS[1], J. M. RADULOVIC[7];
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Abstract: Episodic memory is a capacity to remember autobiographical records of personal events, as well as their relations in time and spatial context in which they occurred. It has been widely accepted that excitatory neurotransmission in the dorsal hippocampus (DH) is a crucial mechanism for the formation of contextual and temporal associative episodic-like memories, which, in mice, have been studied using trace fear conditioning (TFC). In this paradigm the

animal learns to associate a conditioned stimulus such as footshock with a non-conditioned stimulus, such as tone, separated by a temporal trace. In addition to DH, formation of associative memories requires transmission to different neocortical areas, including the retrosplenial cortex (RSC). We previously showed that DH→RSC projections are comprised of two molecularly distinct populations. Here we used a chemogenetic approach to show that, while both vGlut1- and vGlut2-containing DH→RSC terminals redundantly contribute to the formation of tone-shock associations, only vGlut2-containing DH→RSC terminals are necessary for processing the temporal trace. Moreover, using a proteomic strategy based on *in vivo* biotin identification we show that there is a greater protein dynamic in vGlut2- relative to vGlut1-containing projections both following the TFC and under baseline conditions, with vGlut2 terminals showing up-regulation of GABAergic synapses related proteins. Furthermore, we show that inhibition of Gad2 positive RSC interneurons resulted in impaired freezing to temporal trace. Taken together, these data indicate that a feed forward inhibition via vGlut2-containing DH→RSC terminals could be the underlying mechanism for trace coding. Hence, this molecular and functional differentiation of DH→RSC projections could be contributing to separation of the real fear inducing stimuli from the expectation of their occurrence, a process that may be importantly involved in processing of stress-related association of contextual and temporal cues affected in patient populations suffering from anxiety disorders.

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Poster

242. Learning and Memory: Cortical-Hippocampal Interactions I

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant MH119102

Title: Hippocampal efferents to the lateral septum and retrosplenial cortex differentially mediate memory acquisition and retrieval

Authors: *A. N. OPALKA, D. V. WANG;
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Abstract: Learning and memory involves a large neural network of many brain regions, including the notable hippocampus along with the lateral septum (LS) and retrosplenial cortex (RSC). Previous studies have established that the dorsal hippocampus (dHPC) plays a critical

role during the acquisition and retrieval of episodic memories. However, the role of downstream circuitry from the dHPC, including the dHPC-to-LS and dHPC-to-RSC pathways, has come under scrutiny only recently. Here, we employed an optogenetic approach with contextual fear conditioning in mice to determine whether the above two hippocampal efferent pathways are differentially involved in acquisition and retrieval of contextual fear memory. We found that inhibiting the dHPC neuronal terminals to the LS during either acquisition or retrieval impaired subsequent memory performance. On the other hand, inhibiting the dHPC neuronal terminals to the RSC during acquisition impaired subsequent memory performance, whereas inhibition of the same pathway during retrieval had no effect on memory performance. These results indicate that the dHPC-to-LS and dHPC-to-RSC neural pathways play dissociable roles in memory: the former is critical during both memory acquisition and retrieval, while the latter is critical during memory acquisition. The specificity of each pathway reveals the intricacies of memory processing and that multiple hippocampal efferents may be differentially involved in aspects of physiological and cognitive memory processes.

Disclosures: **A.N. Opalka:** None. **D.V. Wang:** None.

Poster

242. Learning and Memory: Cortical-Hippocampal Interactions I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 242.05/W28

Topic: H.01. Animal Cognition and Behavior

Support: CA 224672
RP180055

Title: Docetaxel-induced impairments in visuospatial cognition and executive function

Authors: *A. M. ASHER[1], D. A. MORILAK[2];
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Abstract: Anti-neoplastic drugs are mainstay in the treatment of cancers. However, lasting and profound cognitive impairments relating to visuospatial and verbal memory, attention, and executive function have been reported in 35% of patients following chemotherapy. Mechanisms underlying these cognitive impairments remain unknown. Docetaxel is an effective first line or adjuvant treatment for a variety of cancers, including breast, non-small lung, prostate, and cancers of the head and neck. Docetaxel binds to the positive end of mature microtubules, promoting polymerization and preventing depolymerization, ultimately leading to apoptosis. In neurons, microtubule dynamics have been shown to be important in dendritic spine remodeling during synaptic plasticity, a mechanism thought to underlie learning and memory, and other

cognitive processes. In this study, male and female Sprague-Dawley rats were treated with a low dose of docetaxel (6 mg/kg; i.p.) one time per week for three weeks. Two weeks after the last injection, the novel object location task and the attentional set-shifting task were utilized to assess visuospatial cognition, mediated in the hippocampus (Hipp), and executive function, specifically cognitive flexibility, mediated in the medial prefrontal cortex (mPFC), respectively. Additionally, dendritic spine density and morphology were characterized using DiOlistic labeling of neurons in the mPFC, Hipp, and medial dorsal thalamus (MDT). Functional circuit activity was assessed by measuring afferent-evoked field potential responses in the mPFC to stimulation of the ventral Hipp or MDT. Preliminary data indicate a trend showing visuospatial impairment on the novel object location test following docetaxel treatment, which does not appear to be attributable to non-specific effects on locomotor activity or anxiety-like behavior, assessed on the open field test. Ongoing experiments will assess docetaxel-related effects on mPFC-mediated cognitive flexibility using the attentional set shifting task, as well as dendritic spine density and morphology.

Disclosures: **A.M. Asher:** None. **D.A. Morilak:** None.

Poster

242. Learning and Memory: Cortical-Hippocampal Interactions I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 242.06/W29

Topic: H.01. Animal Cognition and Behavior

Support: College of Arts and Sciences, University of San Diego
McNair Scholars Program, University of San Diego

Title: Effects of medial entorhinal cortex lesions in rats on the traveling salesman problem

Authors: *L. R. OLIVAS, E. A. PETTY, R. BLASER, J. B. HALES;
Psychological Sci., Univ. of San Diego, San Diego, CA

Abstract: While highly constrained behavioral measures are beneficial for isolating and targeting specific processes, they can restrict our understanding of these behaviors in natural settings. The Traveling Salesman Problem (TSP) is a spatial navigational task that differs from many other behavioral tasks because rather than examining an animal's ability to perform a specific behavior, it explores foraging behaviors in a naturalistic setting. Although foraging is a spontaneous behavior, it is also complex, in that it involves decision-making, attention, spatial processing and navigation, course planning, and memory. Our study examined the role of the medial entorhinal cortex (MEC) in the TSP task. Previous research from our lab found that rats with hippocampal lesions were impaired on certain TSP measures, including making more errors by revisiting targets, taking longer to complete the task, and using routes that were less optimal

compared to the sham rats. Experiments utilizing oversimplified conditions have shown that the MEC plays an important role in spatial processing and spatial memory in ways that are similar to, and yet distinct from, those of the hippocampus. Therefore, comparing the effects of MEC lesions to those of hippocampal lesions while rats are performing the TSP task can shed light on the relative contributions of these different anatomical brain areas to naturalistic foraging behavior.

Disclosures: **L.R. Olivas:** None. **E.A. Petty:** None. **R. Blaser:** None. **J.B. Hales:** None.

Poster

242. Learning and Memory: Cortical-Hippocampal Interactions I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 242.07/W30

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant T32 DA037202
NSF Grant IOS1353137

Title: Lesions of the retrosplenial cortex impair unimodal sensory preconditioning

Authors: *D. I. FOURNIER, R. R. MONASCH, T. P. TODD, C. P. PUSKAS, D. J. BUCCI; Psychological and Brain Sci., Dartmouth Col., Hanover, NH

Abstract: The retrosplenial cortex (RSC) is positioned at an interface between cortical sensory regions and the structures that compose the medial temporal lobe memory system. One theory posits that the RSC contributes to learning and memory by forming associations between neutral sensory stimuli in an environment (stimulus-stimulus, or S-S learning). To test the role of the RSC in S-S learning, we have studied the effects of RSC lesions and inactivations in a behavioral procedure referred to as sensory preconditioning. Sensory preconditioning is a rigorous test of S-S learning since it requires associating two neutral cues without any reinforcement. In the first phase of the procedure, two cues (S1 and S2) are serially presented and a third cue (S3) is presented alone. In the subsequent conditioning phase, S2 is paired with food reinforcement. In the final test phase, rats received presentations of S1 and S3. Rats that had formed an association between S1 and S2 exhibit more food cup responding during presentation of S1 compared to S3. In our previous studies, we found that permanent lesions of RSC, or temporary inactivation of RSC only during the first phase of training, impair the sensory preconditioning effect when multimodal sensory stimuli are used (i.e. S1 and S3 were auditory stimuli and S2 was a visual stimulus). Here we tested whether RSC would also be important for sensory preconditioning using unimodal stimuli. To that end, S1, S2, and S3 were all auditory (A) stimuli. We found that sham-lesioned rats responded more to A1 than A3, indicative of the standard sensory preconditioning effect. In contrast, either electrolytic and neurotoxic lesions of RSC rats

eliminated the effect, in that rats in those groups exhibited comparably low levels of responding to both A1 and A3 during the test session. These results indicate that the RSC contributes to the S-S learning involving both multisensory and unimodal discrete stimuli.

Disclosures: D.I. Fournier: None. **R.R. Monasch:** None. **T.P. Todd:** None. **C.P. Puskas:** None. **D.J. Bucci:** None.

Poster

242. Learning and Memory: Cortical-Hippocampal Interactions I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 242.08/W31

Topic: H.01. Animal Cognition and Behavior

Support: scholarship 629957 CONACYT
UNAM-PAPIIT IN224019

Title: Hippocampal-dependent spatial memory impairment in acute and chronic hyperglycemic mice

Authors: *D. I. DEL MORAL[1], T. MOLINA-JIMÉNEZ[2], C. J. JUÁREZ-PORTILLA[3], T. MEZA-MENCHACA[4], F. GARCÍA-ORDUÑA[5], M. FLORES-MUÑOZ[6], Ó. LÓPEZ-FRANCO[6], R. C. ZEPEDA[3], G. R. ROLDAN[7];
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Abstract: Type 1 and type 2 diabetes have been associated with learning and memory impairment. Thus, pediatric and adult diabetic patients showed cognitive deficits; reduced intellectual performance and neurophysiological studies demonstrated neurological changes. Despite the large number of studies in human diabetic patients and diabetes syndrome animal models, there is no consensus about the mechanisms that underlie cognitive dysfunction during hyperglycemia. Preclinical studies usually report deficits in learning and memory test performances, but the results seems to differ according to the diabetes type model and learning paradigms used. To elucidate the differences between acute and chronic effects of hyperglycemia on learning and memory, we used two hyperglycemia models: 1) Chronic hyperglycemia model, based on the consumption of high fat and carbohydrates diet (HFCD), and 2) acute hyperglycemia model using Streptozotocin (STZ), both in eight-week old male CD-1 mice. In model 1, mice were fed with the HFCD and 10% sucrose water during 12 weeks. Control group received commercial pellets and tap water. After this period, the animals were evaluated using

the eight-arm maze, with rewards in 4 arms. In model 2, mice were administered with a single dose of 100 mg/kg of STZ i.p., and after two weeks tested in the eight-arm maze. Control group received vehicle. All sessions were videotaped and the latency to find and eat each reward, as well as the number of errors in working (when the mouse re-enter in one arm) and reference (when the mouse enters the arms without food) memory were quantified. The analyses were performed using ImageJ Pro plus, v. 5 (Media Cybernetics, Silver Spring, MD, USA). For the analysis of the data, a design of fixed and nested factors with a generalized linear model adjustment was applied. The results showed memory deficits in hyperglycemic mice compared to control animals in both models. We conclude that persistent hyperglycemia affect hippocampal-dependent spatial memory.

Disclosures: D.I. Del Moral: None. **T. Molina-Jiménez:** None. **C.J. Juárez-Portilla:** None. **T. Meza-Menchaca:** None. **F. García-Orduña:** None. **M. Flores-Muñoz:** None. **Ó. López-Franco:** None. **R.C. Zepeda:** None. **G.R. Roldan:** None.

Poster

242. Learning and Memory: Cortical-Hippocampal Interactions I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 242.09/W32

Topic: H.01. Animal Cognition and Behavior

Support: NINDS IRP (ZIA NS003168)

Title: Closed-loop sinusoidal stimulation of ventral hippocampal terminals in prefrontal cortex preferentially entrains circuit activity at distinct frequencies and delays

Authors: *M. V. M. MYROSHNYCHENKO[1], D. A. KUPFERSCHMIDT[2], J. A. GORDON[3]; [1]Natl. Inst. of Neurolog. Disorders and Stroke, BETHESDA, MD; [2]Integrative Neurosci. Section, Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD; [3]Office of the Director, Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Dynamic changes in oscillatory synchrony of ventral hippocampus (vHPC) and prefrontal cortex (PFC) correlate with various cognitive functions. Optogenetic inhibition of discrete neuronal projections from vHPC to PFC has been shown in mice to disrupt vHPC-PFC synchrony and cognitive function. Lacking, however, is an understanding of how oscillatory synchrony of discrete neuronal projections contributes to broader circuit dynamics and cognition. Here we used both open- and closed-loop optogenetic manipulations of oscillatory activity in vHPC-PFC projections to identify (1) properties of vHPC-PFC circuit dynamics and (2) novel approaches to controlling vHPC-PFC oscillatory synchrony. Local field potential and single-unit recordings were made from mouse vHPC and PFC during sinusoidal optogenetic stimulation of vHPC inputs to PFC. Open-loop sinusoidal stimulation at frequency ranges centering on 8 Hz

and 25-35 Hz maximally enhanced local field potential power in PFC and vHPC, respectively. Closed-loop sinusoidal stimulation that mimicked real-time frequency-filtered oscillations of the vHPC field potential was also capable of entraining PFC and HPC activity; however, the degree of entrainment depended strongly on the phase delay implemented in the feedback procedure. Delaying optical stimulation relative to a given vHPC hippocampal oscillation by approximately half of its period caused robust entrainment of PFC and vHPC field potentials to the stimulation frequency and increased phase-locking of PFC unit spiking to vHPC field potentials. In contrast, closed-loop stimulation implemented with no phase delay resulted in only modest oscillatory entrainment. These results stand to inform computational models of communication between vHPC and PFC, and guide the use of continuously varying, closed-loop stimulation to assess effects of enhancing endogenous long-range neuronal communication on behavioral measures of cognitive function.

Disclosures: **M.V.M. Myroshnychenko:** None. **J.A. Gordon:** None. **D.A. Kupferschmidt:** None.

Poster

242. Learning and Memory: Cortical-Hippocampal Interactions I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 242.10/W33

Topic: H.01. Animal Cognition and Behavior

Title: The interaction between hippocampus and temporal cortex during memory consolidation and recall

Authors: *S. TAKAMIYA, S. YUKI, J. HIROKAWA, Y. SAKURAI;
Doshisha Universuty, Kyotanabe-shi, Japan

Abstract: Cell assemblies are populations of functionally connected neurons that encode memory and can be memory engrams. Recently, experiments utilizing optogenetics revealed reactivation of engram cells in hippocampus is necessary for retrieval of “recent” memory while in neocortex is necessary for retrieval of “remote” memory. However, these experiments used behavioral tasks that can be learned in only one experience, such as contextual fear conditioning. Therefore, the activation and reactivation of engram cells which gradually encode memory over a longer time span is unclear. The objective of this study is to reveal the dynamic change of cell assemblies in gradual processes of memory consolidation and rapid processes of memory recall using a complex behavior task and multineuronal recording. We used a conditional discrimination task with tones where rats were required to discriminate between high and low pitches. Multineuronal activities were recorded from the hippocampus and temporal cortex during the learning processes of the task. After an interval following completion of learning, we retrained the rats with the same behavioral task to make them recall the memory of the task. In

the processes of learning and recalling, we detected changes in neuron activities. We discuss the relation between dynamic activities of neurons in the hippocampus and temporal cortex and degrees of memory consolidation and recall , and the interaction between hippocampus and temporal cortex.

Disclosures: **S. Takamiya:** None. **S. Yuki:** None. **J. Hirokawa:** None. **Y. Sakurai:** None.

Poster

242. Learning and Memory: Cortical-Hippocampal Interactions I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 242.11/W34

Topic: H.01. Animal Cognition and Behavior

Support: EU Marie Curie Fellowship 753608
EU ERC Grant 639272
NFR Grant 231495

Title: Cell type-specific responses in the neocortex during hippocampal sharp wave ripples

Authors: *A. R. CHAMBERS, K. VERVAEKE;
Inst. of Basic Med. Sci., Univ. of Oslo, Oslo, Norway

Abstract: Memory recall and consolidation likely involve a bidirectional dialogue between the hippocampus and neocortex. This is thought to take place during high-frequency oscillatory events (sharp-wave ripples, SWR) in the CA1 region of the hippocampus that occur during sleep and quiet wakefulness. SWR-related re-activation of neural ensembles that were previously active while awake has been implicated in the long-term storage of memory traces in a distributed neocortical network. However, the relationship between neocortical activity and hippocampal SWR is not well understood at the mechanistic level. Inhibitory neurons, specifically, could play a prominent role in SWR-associated neocortical processing because they control the propagation of excitation through circuits. To potentially facilitate memory-related plasticity and the reactivation of weakly connected neural ensembles, we hypothesized that inhibitory neurons could modulate their activity during SWR in order to increase neocortical excitability during brief periods around SWR. We performed two-photon calcium imaging of neurons in sensory (visual and somatosensory) and association (parietal and retrosplenial) areas of the neocortex while recording SWR in the ipsilateral hippocampus. To identify cell-type specific responses, we used mouse lines and genetically encoded calcium indicators (Thy1-GCaMP6s for excitatory neurons, and Gad2-, VIP-, SST-, and PV-Cre recombinase expressing mice to target inhibitory neurons with virally delivered GCaMP6s). Experiments were performed while animals were awake in the dark, and allowed to walk or rest at-will on a disc. In both excitatory and inhibitory neuron populations, a mix of pre-SWR ramping suppression and peri-

SWR sharp activation was found. In cortical layer 1, where inhibitory interneurons exert influence over the tuft dendrites of pyramidal neurons, the most prominent response (10-20% of all cells) consisted of a ramping suppression of activity starting approximately 1.5-2 seconds before the SWR in the hippocampus. This response in layer 1 was observed in both sensory and association areas with similar proportions, and the degree of suppression was correlated with ripple amplitude. Other cell populations in deeper layers showed mixed responses: Thy1, VIP, and PV neurons in sensory and association regions could show either a similar slow suppression or a fast activation that peaked at the ripple peak, and SST neurons showed only activation. Ongoing experiments aim to identify the mechanisms of the inhibitory neuron suppression, and to determine whether this provides an essential window of higher neocortical excitability during SWR.

Disclosures: A.R. Chambers: None. **K. Vervaeke:** None.

Poster

242. Learning and Memory: Cortical-Hippocampal Interactions I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 242.12/W35

Topic: H.01. Animal Cognition and Behavior

Support: KAKENHI 17H02221
KAKENHI 16H01888

Title: Postnatal stimuli modulate cortico-hippocampal network dynamics

Authors: *Y. SHINOHARA[1], S. KOKETSU[2], H. HIRASE[4], T. UEKI[3];
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Abstract: Phase-locked synchronization between the cerebral cortex and hippocampus has been implicated to be important during working memory and spatial navigation. However, how animal experience organizes cortico-hippocampal dynamics is largely unknown. To analyze synchronized activity between the hippocampus and various cortical areas, we used transgenic mice (G7NG817), which expresses the calcium indicator G-CaMP7 in astrocytes and the majority of excitatory neurons in the cortex. The mice allow us to observe the temporal dynamics of cortical calcium in 25-100Hz transcranially. Environmental effects on cortico-hippocampal dynamics were observed by comparing mice reared under two distinct conditions, either in enriched environment (ENR) or isolated condition (ISO). After 4 weeks rearing, we measured cortical calcium dynamics and hippocampal local field potentials simultaneously. We found that the cortical calcium dynamics was closely correlated to hippocampal EEG status in urethane anaesthetized mice. Regardless of the rearing conditions, calcium activities observed during theta

and non-theta states showed distinct spatial and temporal patterns. Cortical calcium level is higher in wide areas of the cortex during the theta state, and the temporal calcium fluctuation is relatively mild. During non-theta states, basal calcium level is lower, but larger cortical calcium elevations that spread over cortical areas co-occurred with hippocampal sharp wave and ripple (SWR) oscillations. Next, we investigated spatio-temporal dynamics between hippocampal SWR events and cortical calcium using head-restrained un-anaesthetized mice monitoring animal sleep/awake states. In both states, calcium activities preceded hippocampal SWR events in vision-related cortical areas. During awake SWRs, wide areas of cortical calcium elevation co-occurred with hippocampal SWRs, while sleep SWRs showed delayed frontal cortical activation after SWRs. Interestingly, ENR mice exhibited sparser activity patterns. Our data indicate that the cortico-hippocampal dynamics show distinct patterns depending on animal states, but postnatal experience also play a role for modulating the network dynamics.

Disclosures: Y. Shinohara: None. **S. Koketsu:** None. **H. Hirase:** None. **T. Ueki:** None.

Poster

242. Learning and Memory: Cortical-Hippocampal Interactions I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 242.13/W36

Topic: H.01. Animal Cognition and Behavior

Support: NIH grant AG 046266
NIH grant S10 OD018132
NSF grant DMR-1157490

Title: Superior cognitive flexibility and associated changes in resting brain networks in male middle-aged marmosets compared to females

Authors: M. LACLAIR[1], M. FEBO[2], B. NEPHEW[3], N. GERVAIS[4], G. POIRIER[5], K. WORKMAN[7], *C. M. MOORE[6], J. A. KING[3], A. LACREUSE[7], **R. J. CALI**[5];
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Abstract: Sex differences in human cognitive performance are well characterized. However, the neural correlates of these differences remain elusive. This issue may be best studied in nonhuman primates, for which sociocultural influences are minimized. We used the marmoset (*Callithrix jacchus*) to investigate sex differences in two aspects of executive function: Reversal Learning and Intradimensional/Extradimensional (ID/ED) set shifting. Stress reactivity,

measured through social separation, and motor function were also assessed. In agreement with human literature, females required more trials than males to acquire the reversals. No sex differences in ED set shifting or motivational measures were observed. The findings suggest enhanced habit formation in females, perhaps due to striatal estrogenic effects. Both males and females showed increased urinary cortisol during social separation, but females showed an earlier increase in cortisol and a greater increase in agitated locomotion, possibly indicating enhanced stress reactivity. No sex differences were found in motor performance. Associations between brain networks and prior reversal learning performance were investigated using resting state fMRI. Resting state functional connectivity analyses revealed sex differences in cognitive networks, with differences in both overall neural network metrics and specific regions, including the prefrontal cortex, caudate, putamen, and nucleus accumbens. Correlations between cognitive flexibility and neural connectivity indicate that sex differences in cognitive flexibility are related to sex-dependent patterns of resting brain networks. Overall, our findings reveal sex differences in cognitive flexibility, brain networks, and their relationship in the marmoset, positioning this species as an excellent model to investigate the biological basis of cognitive sex differences.

Disclosures: **M. LaClair:** None. **M. Febo:** None. **B. Nephew:** None. **N. Gervais:** None. **G. Poirier:** None. **K. Workman:** None. **C.M. Moore:** None. **J.A. King:** None. **A. Lacreuse:** None.

Poster

242. Learning and Memory: Cortical-Hippocampal Interactions I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 242.14/W37

Topic: H.01. Animal Cognition and Behavior

Title: Electrophysiological analysis of neocortical-hippocampal interplay in rats performing a spatially cued object recognition task

Authors: *O. J. RAUHALA[1], J. GELINAS[2], D. KHODAGHOLY[1];
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Abstract: Various neural mechanisms have been implicated in object recognition and identification as cues for navigation and spatial memory. Previous studies have elucidated mechanisms causal to spatially cuing an object to a reward but on the basis of a fairly local neural circuit. We aim to investigate large-scale multi-region neocortical-hippocampal interactions in animals that combine information on object identity, location and association to perform complex navigational behavior.
Our search includes mechanisms that facilitate learning during training but also memory consolidation during subsequent sleep. In addition to the hippocampus (HC), we pay special attention to cortical regions involved in decision making in reward contexts such as the medial

prefrontal cortex (mPFC) and higher order association areas such as the posterior parietal cortex (PPC). Finally, our recording methods also allow us to analyze global neocortical activity. We implant rats with NeuroGrids that cover the dorsal cortical surface of entire hemispheres as well as silicon probes in the HC and mPFC to collect local field potential (LFP) and spiking activity during behavioral training and sleep. We then train the rats to associate a particular object to reward and to dislocate the object to uncover a reward while ignoring non-reward associated objects on the maze. When rats retain multiple object-reward associations, we place two reward-associated objects simultaneously on the maze but only reward one based on its location, thus adding a spatial component. We measure learning and performance as lack of incorrect object dislocations.
The key focus of our investigation is the spatiotemporal interactions of the HC with diverse functional cortical regions as well as the interactions between these cortical regions at all online and offline states of the task. Acquiring and analyzing neural LFP and spiking activity simultaneously from multiple functionally distinct brain regions in multi-modal behavior provides valuable insight to how multiple types of information, here 'what' and 'where', are processed and consolidated in the brain in tandem.

Disclosures: O.J. Rauhala: None. **J. Gelinas:** None. **D. Khodagholy:** None.

Poster

242. Learning and Memory: Cortical-Hippocampal Interactions I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 242.15/W38

Topic: H.01. Animal Cognition and Behavior

Title: Early infant experiences shape the adult allocentric spatial performance and the associated functional brain circuitry in rats

Authors: *M. P. CONTRERAS[1,3], M. MENDEZ[4], J. BORN[1,2], M. INOSTROZA[1];
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Abstract: Episodic memories are genuinely dependent on the hippocampal memory system. Episodic memories encoded during early infancy are thought to be rapidly forgotten, a phenomenon known as infantile amnesia. Nevertheless, early experiences are commonly assumed to systematically influence adult behavior, raising the question in which manner infantile memories affect adult behavior. In our study, we ask whether a training on a task that demands hippocampal function (compared with training on a non-hippocampal task) during infancy influences the adult capacity to form allocentric spatial representations as well as the

associated functional brain circuitry. To address this question, we evaluated three different groups of infant rats undergoing training on a hippocampal task, training on a non-hippocampal task, and no experimental training, respectively. Later, in adulthood, we tested their allocentric spatial performance. The training on the hippocampal task during early infancy consisted of four runs on a classical object recognition (OPR) tasks with a 5 min retention interval between encoding and retrieval phase, and with different objects on each run. The four runs were performed on each second day, with the first run taking place on postnatal day 18. The non-hippocampal task training was addressed by using the novel-object recognition task on the same days and time than the previous group. The "no experience" group did not receive any task training during early infancy. At the adulthood testing, all the rats were subjected to the OPR task, again with different objects and, here, using a 3-hours retention interval between encoding and retrieval phase. Ninety minutes after the retrieval test brains were removed for immunocytochemistry analyses of c-fos and quantitative cytochrome c oxidase (COx). We found that adult spatial performance was critically shaped depending on the type of early experience, i.e., the rats subjected to training on the hippocampal-dependent task during early childhood showed enhanced capabilities of spatial discrimination on the OPR at the test in adulthood, and this was associated with a particular pattern of c-fos activity in a prefrontal-thalamic-hippocampal functional brain network.

Disclosures: M.P. Contreras: None. **J. Born:** None. **M. Inostroza:** None. **M. Mendez:** None.

Poster

242. Learning and Memory: Cortical-Hippocampal Interactions I

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Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 242.16/W39

Topic: H.01. Animal Cognition and Behavior

Support: NIH 1R24MH109060-01

Title: Activation of hippocampal CA2 region precedes CA3 following perforant-path stimulation and spontaneously occurring dentate spikes

Authors: *C. A. WILHITE[1], R. S. WITTE[1], S. L. COWEN[2];
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Abstract: Memory functions rely on precise signal processing through the entorhinal-hippocampal network, which includes the entorhinal cortex (EC), dentate gyrus (DG), and hippocampus proper (CA1, CA2, CA3). Most previous studies investigating signal transfer through this network have focused on the trisynaptic circuit (EC -> DG -> CA3 -> CA1). Although the trisynaptic circuit and associated hippocampal regions (DG, CA3, CA1) have been extensively studied, the role of CA2 in the processing of signals within the EC-hippocampal

network remains unclear *in vivo*. Here we used high-density silicon arrays to analyze evoked potentials and large-scale network patterns across the dorsal hippocampus (DG, CA3, CA2, CA1) in anesthetized (n = 3) and chronically implanted (n = 2) rats. In the anesthetized procedures, local-field potentials were acquired in response to electrical stimulation of the medial entorhinal afferents to the hippocampus (medial perforant-path). We found that the peak current source density (CSD) response in CA2 preceded the peak response in CA3 (Student's t-test, $p < 1.2$ e-15, n = 120 stimulations). Cross-correlation measures revealed a > 2 ms lag between peak CSD responses in CA2 and CA3. Furthermore, the amplitude of the peak CSD in CA2 was significantly greater than the peak CSD in CA3 (Student's *t*-test, $p < 2.2$ e-15). Strong activation of the hippocampus is also observed during rest in the form of dentate spikes, which are high-amplitude, short-latency local-field responses thought to reflect coordinated EC input. We found that the peak CSD response in CA2 preceded the peak response in CA3 during dentate spikes compared to both pre- and post-dentate spike intervals (1-way ANOVA, p values < 3 e-4, n = 87 events). Cross-correlation measures revealed a > 1 ms lag between peak CSD responses in CA2 and CA3. Moreover, the amplitude of the peak CSD in CA2 was significantly greater than the peak CSD in CA3 during dentate spikes (Student's *t*-test, p= < 6 e-39). These results demonstrate a powerful transfer of signals from EC to hippocampal CA2 *in vivo* that runs in parallel with the classical tri-synaptic circuit. These data suggest a key role of area CA2 in the initial processing of cortical input within the EC-hippocampal memory network.

Disclosures: C.A. Wilhite: None. **R.S. Witte:** None. **S.L. Cowen:** None.

Poster

242. Learning and Memory: Cortical-Hippocampal Interactions I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 242.17/W40

Topic: H.01. Animal Cognition and Behavior

Support: FONCYT-PICT 2014-3155
SECYT-Universidad Nacional de Cordoba

Title: Granular subdivision of retrosplenial cortex (A29) is critically required for retrieval of contextual threatening memories

Authors: *S. DE OLMOS, E. SIGWALD, A. LORENZO;
Inst. de Investigacion Medica Mercedes y Martin Ferreyra-INIMEC-CONICET-Universidad Nacional de Cordoba, Cordoba, Argentina

Abstract: The contribution of the retrosplenial cortex (RSC) to contextual fear memory (CFM) is increasingly recognized, however the role of the granular (area 29, A29) and dysgranular (area 30, A30) subdivisions of the RSC remains elusive. Here, we performed comprehensive

behavioral studies to dissect the contribution of A29 to CFM retrieval. To that end, orchiectomized (ORC) and intact male rats received an intraperitoneal (I.P.) injection of saline (control) or 5mg/Kg MK801 after training and memory formation. Previously, we showed that in orchiectomized (ORC), but not in intact male rats, this MK801 treatment induces overt loss of neurons selectively in layers IV-Va of A29 ($A29^{MK801}$ neurons), and impairs freezing expression during CFM retrieval (Sigwald et al., Brain Struct Funct 2016). Here, we show that, compared to ORC-saline, ORC-MK801 rats showed impaired CFM retrieval in an A-B-A design for contextual-fear conditioning (ANOVA $F(1,29) = 3.6834$, $p=0.004$); however, context discrimination was not affected. In ORC animals, neither novel object recognition (ANOVA $F(1,14)=0.00002$, $p = 0.996$) and object-in-context discrimination (ANOVA $F(1,11)=0.63115$, $p = 0.44$) were impaired by MK801, further indicating that loss of $A29^{MK801}$ neurons do not affect contextual discrimination. Elevated plus maze test showed that anxiety-like behavior was not affected in ORC-MK801 animals (ANOVA $F(1,12)=0.2498$, $p = 0.6262$), suggesting that loss of $A29^{MK801}$ neurons does not affect the emotional state that could impair freezing during test. Importantly, in a sensory preconditioning test (ANOVA ($F(1,17)=4.7184$), MK801 treatment abolished higher order CFM retrieval in ORC, but not in intact male rats; which indicates that loss of $A29^{MK801}$ neurons severely impairs higher order memory retrieval. Collectively, our data provides the first evidence indicating that neurons in layers IV-Va of A29 are critically required for the retrieval of an associative memory, however are dispensable for context discrimination or modulating the emotional state of the animal. The anatomofunctional mechanism by which $A29^{MK801}$ neurons could participate in CFM retrieval will be discussed.

Disclosures: S. de Olmos: None. **E. Sigwald:** None. **A. Lorenzo:** None.

Poster

242. Learning and Memory: Cortical-Hippocampal Interactions I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 242.18/W41

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant K12HD073945
CNT Grant EEC-1028725
NIH Grant R01 NS102886
VA Merit Award I01BX003335

Title: Changes in cortico-hippocampal functional networks as a result of stroke-induced diaschisis

Authors: *Z. IP[1], G. RABILLER[3], J. HE[3], Z. YAO[1], Y. AKAMATSU[3], Y. NISHIJIMA[3], J. LIU[3], A. YAZDAN-SHAHMORAD[2];

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Abstract: Stroke is the leading cause of disability globally; 15 million people suffer from stroke worldwide each year. The functional sequelae following cortical stroke encompass not only motor but also cognitive impairment including memory function. However, the infarct zone of cortical stroke rarely extends to the hippocampus, which is well known for its role in learning and memory function. The functional networks connecting the hippocampus and cortex and how they are affected following chronic stroke is currently not well understood.
To interrogate these functional networks, we induced ischemic stroke in the left somatosensory cortex of rats via distal occlusion of the middle cerebral artery (dMCAO) and used linear micro-electrode arrays to simultaneously record local field potentials from sensory-motor cortex and various hippocampal layers under urethane anesthesia two weeks and one-month post stroke. We analyzed the functional connections between cortical and hippocampal layers by investigating changes to signal power ratios between frequency bands and hemispheres, cross-frequency coupling, and sharp-wave ripples. Our results show that after stroke, the signal power ratio of high-gamma band in the ipsilateral hemisphere significantly increases when compared to the contralateral hemisphere in both cortex and hippocampus. The stability of theta/delta states is disrupted, and modulation of cortical gamma by hippocampal theta changes. In the low theta/delta state, the occurrence and amplitude of the sharp-wave ripples significantly increases ipsilaterally.
Despite the infarcted area being limited to the sensory-motor cortex, stroke can have a far-reaching effect on remote areas by disrupting functional dynamics. Hippocampal diaschisis arises as a result of cortical stroke. Further understanding of cortico-hippocampal circuits may reveal novel strategies for diagnosis and detection of stroke, as well as possible therapies for restoring function through cortical rewiring of networks by paired stimulation.

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Poster

242. Learning and Memory: Cortical-Hippocampal Interactions I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 242.19/W42

Topic: H.01. Animal Cognition and Behavior

Title: Arc mRNA quantification in CA1 and perirhinal/lateral entorhinal cortex during weak and strong object memory consolidation in male C57BL/6J mice

Authors: *D. A. CINALLI, Jr[1], S. J. COHEN[2], P. A. GAJEWSKI-KURDZIEL[4], R. W. STACKMAN, JR[3];

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Abstract: Arc, an effector immediate early gene, has been linked to experience-dependent synaptic plasticity and plays a key role in the consolidation of episodic memory. It is hypothesized that the perirhinal cortex and CA1 region of dorsal hippocampus play complementary, but dependent, roles in the encoding and consolidation of object memory in rodents. Here we used quantitative polymerase chain reaction (qPCR) to quantify Arc mRNA expression within the CA1 region of dorsal hippocampus and in perirhinal cortex (PER)/lateral entorhinal cortex (LEC) of adult male C57BL/6J mice during object memory consolidation 40 min post-sample session exploration in the spontaneous object recognition (SOR) task under either weak or strong memory load conditions. Mice were placed into a familiar high-walled square arena that contained two identical novel objects during the sample session. A weak memory load was defined as having explored each object for 10 s, while strong memory load was defined as having explored each object for 30s. QPCR samples were taken bilaterally from PER/LEC and CA1 with a 1 mm diameter tissue punch. Previous work in our lab has shown that temporary inactivation of CA1 during encoding, consolidation or retrieval results in impairment in strong memory SOR, but not in weak memory SOR. Conversely, inactivation of PER during weak memory consolidation resulted in significant impairment, while inactivation of PER during strong memory consolidation showed no impairment. We also tested whether inactivation of PER would impair weak and strong object memory encoding and retrieval by locally infusing muscimol in PER prior to sample session or prior to test session in both weak and strong memory conditions. Together, results from these studies along with previous work in our lab improve the understanding of the interaction of the hippocampus and associated medial temporal lobe cortical areas in object memory processing.

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Poster

242. Learning and Memory: Cortical-Hippocampal Interactions I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 242.20/W43

Topic: H.01. Animal Cognition and Behavior

Support: Whitehall Grant
Harlan Scholars

Title: Acetylcholine supports EC-CA1 coupling by suppressing competing drivers

Authors: *D. M. LAYFIELD[1], J. HERNÁNDEZ[2], B. SCHRITTER[1], E. L. NEWMAN[3]; [2]Dept. of Psychological and Brain Sci., [1]Indiana Univ., Bloomington, IN; [3]Dept. of Psych. and Brain Sci., Indiana Univ. Bloomington, Bloomington, IN

Abstract: Ongoing behavior requires rapid and persistent cognitive flexibility which emerges from changes in the functional connectivity of the brain's networks. The direct projection from the entorhinal cortex (EC) to CA1 of the hippocampus (HPC) is theorized to gate encoding of new information. The mechanisms regulating coupling between these areas are not well characterized. Here, we test the hypothesis that local acetylcholine release modulates the functional coupling between these areas. To test this hypothesis, we examined the influence of stimulating acetylcholine release on the functional coupling between EC and CA1 in freely behaving rats. To stimulate acetylcholine release, we virally induced channelrhodopsin expression in acetylcholine producing neurons of the medial septum by injecting AAV5-EF1a-DIO-hChR2(H134R)-EYFP locally in ChAT-CRE rats. To track functional coupling, we examined theta-gamma coupling in stratum lacunosum-moleculare (LM) of CA1. Stimulation and recordings were performed in a variant of the novel-object-in-place task. Preliminary results show that stimulating acetylcholine release increased phase-amplitude coupling between mid-gamma and theta in LM. This increase occurred despite the reduction in mid-gamma amplitude. These results support the hypothesis that acetylcholine modulates functional coupling between EC and CA1. Specifically, they indicate that cholinergic gating is unlikely to be induced by overtly engaging, or strengthening, a specific process. Rather, we suggest that acetylcholine restricts or dampens competing circuit dynamics, thereby increasing the relative dominance of the entorhinal input.

Disclosures: D.M. Layfield: None. **J. Hernández:** None. **B. Schritter:** None. **E.L. Newman:** None.

Poster

242. Learning and Memory: Cortical-Hippocampal Interactions I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 242.21/W44

Topic: H.01. Animal Cognition and Behavior

Support: National Science Foundation [NSF CAREER Award 1565410 (I.A.M)]
National Institutes of Health [NIH, NIGMS, grant GM122645 (A.J.A.)
TSA MARC Program GM007717 (J.H.V.)].

Title: Pathway specific activation of ventral hippocampal cells projecting to the prelimbic cortex diminishes fear renewal

Authors: *J. H. VASQUEZ[1], K.-C. LEONG[2], C. M. GAGLIARDI[1], B. HARLAND[1], A. APICELLA[1], I. A. MUZZIO[1];
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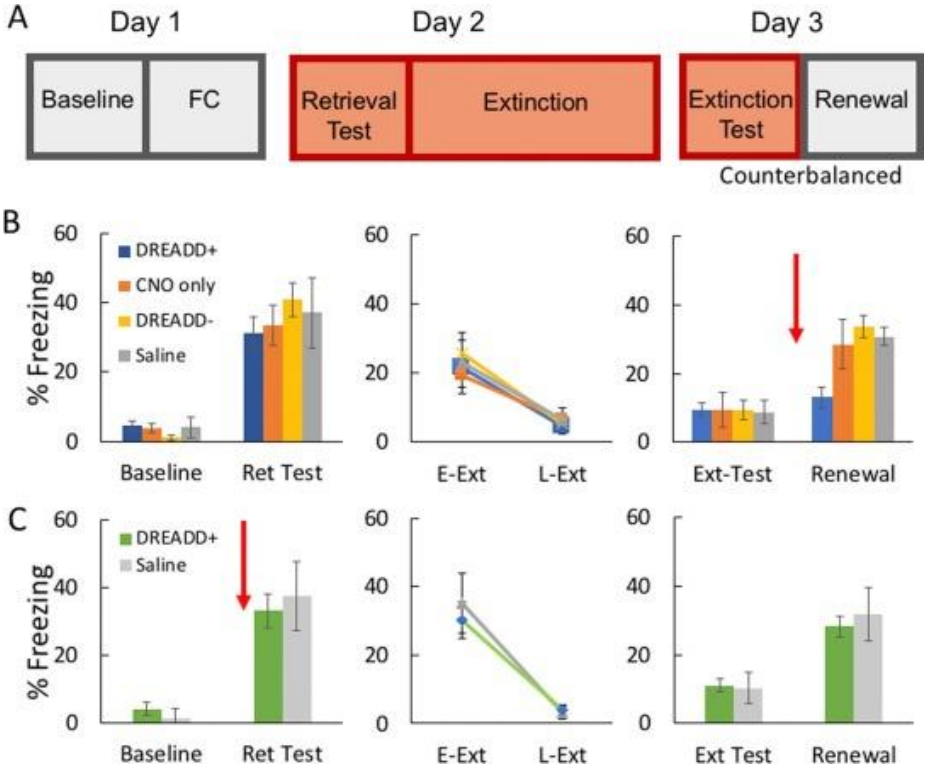
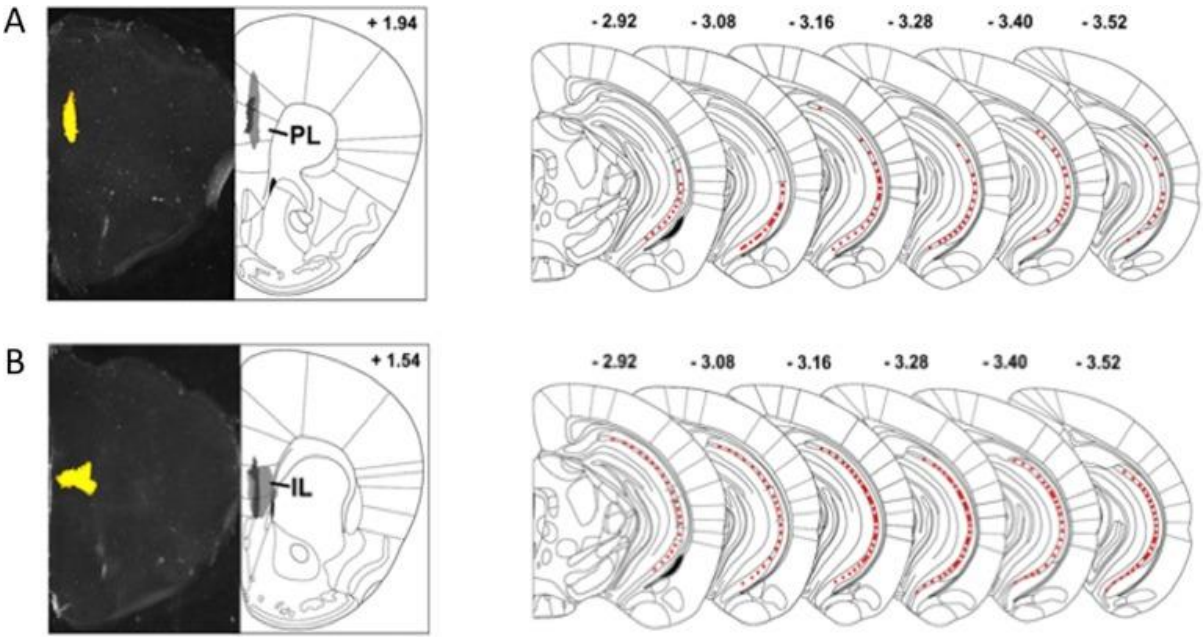
Abstract: The ability to learn that a stimulus no longer signals danger is known as extinction. A major characteristic of extinction is that it is context-dependent, which means that fear reduction only occurs in the same context as extinction training. In other contexts, there is re-emergence of fear, known as contextual renewal. The ability to properly extinguish fear memories and generalize safety associations to multiple contexts provides therapeutic potential, but little is known about the specific neural pathways that mediate fear renewal and extinction generalization. The ventral hippocampus (VH) is thought to provide a contextual gating mechanism that determines whether fear or safety is expressed in particular contexts through its projections to areas of the fear circuit, including the infralimbic (IL) and prelimbic (PL) cortices. Moreover, VH principal cells fire in large, overlapping regions of the environment, a characteristic that is ideal to support generalization; yet it is unclear how different projection cells mediate this process. Using a pathway-specific (intersectional) chemogenetic approach, we demonstrate that selective activation of VH cells projecting to PL attenuates fear renewal without affecting fear expression. These results have implications for anxiety disorders since they uncover a neural pathway associated with extinction generalization.





Disclosures: **J.H. Vasquez:** None. **K. Leong:** None. **A. Apicella:** None. **C.M. Gagliardi:** None. **B. Harland:** None. **I.A. Muzzio:** None.

Poster

242. Learning and Memory: Cortical-Hippocampal Interactions I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 242.22/X1

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant MH113626
NIH Grant ES006189

Title: Behavior-related spectral modes of hippocampal activity comprise distinct delta- and theta-dominated network states, including temporally-evolving 'absence' seizures in rats chronically exposed to lead (Pb2+)

Authors: *N. W. SCHULTHEISS[1], J. L. MCGLOTHAN[2], D. R. BROOKS[2], T. R. GUILARTE[2], T. A. ALLEN[1];
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Abstract: The frequency composition of local field potential (LFP) recordings reflects the synchrony of synaptic interactions within local neuronal populations and amongst inputs originating from distributed networks. Using chronically-implanted 32 channel fixed-electrode probes, we recorded LFPs from dorsal CA1 of the hippocampus (HC) of 22 Long-Evans rats, 13

of which were chronically exposed to lead (Pb2+) during development. Recordings were made while rats explored an open field (120x120 cm) or foraged for food distributed sporadically. Combining time-frequency decomposition and hierarchical clustering, we derived putative modes of HC activity based on the spectral content of LFPs during ~200 recordings sessions (30-250 min each). We focused our preliminary analyses on delta-band (1-4 Hz) activity, thought to reflect thalamic input from nucleus reuniens, and on theta-band (5-11 Hz) activity coordinated by medial septal activity as well as intrinsic mechanisms. Consistent with previous reports, HC theta was strong during running for all animals, and instantaneous theta frequency and amplitude were correlated with running speed. Conversely, HC delta was strong when animals were stationary. Interestingly, we sometimes observed sporadic episodes of strong theta accompanied by several theta-harmonic bands when animals were not making any measurable movements. These episodes reproduced precisely the defining features of nonconvulsive 'absence' seizures, and administration of ethosuximide (ETX) (50-200 mg/kg), a first-choice drug for absence epilepsy, eliminated the events. Preliminary comparisons of these seizure-like events between groups suggests an exacerbation of epileptic activity in the Pb2+-exposed rats relative to controls. Next, by varying spectral analysis and clustering parameters we were able to precisely characterize the temporal evolution of seizure activity from transient low-power at a relatively high frequency (~11 Hz) to sustained power at ~8Hz lasting 3-30 seconds. Cross-correlational analysis between spectral modes suggested elevated delta activity could predict impending seizures. Furthermore, the possibility that developmental Pb2+ exposure exacerbates absence epilepsy motivates further inquiry into the mechanisms that engender susceptibility to entrainment with thalamocortical oscillations during seizures.

Disclosures: N.W. Schultheiss: None. **J.L. McGlothan:** None. **D.R. Brooks:** None. **T.R. Guilarte:** None. **T.A. Allen:** None.

Poster

242. Learning and Memory: Cortical-Hippocampal Interactions I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 242.23/X2

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant MH113626

Title: Two separate populations of medial prefrontal cortex cells project to nucleus reuniens and perirhinal cortex in support of different memory retrieval strategies

Authors: *M. SCHLECHT[1], M. JAYACHANDRAN[2], S. B. LINLEY[3], S. V. MAHLER[4], R. P. VERTES[5], T. A. ALLEN[2];
[1]Psychology, [2]Dept. of Psychology, Florida Intl. Univ., Miami, FL; [3]Florida Atlantic Univ., Boca

Raton, FL; [4]Neurobio. and Behavior, Univ. of California Irvine Dept. of Neurobio. and Behavior, Irvine, CA; [5]FAU/Ctr Complex Systems, Boca Raton, FL

Abstract: The medial prefrontal cortex (mPFC) is ideally situated to influence episodic memory retrieval through its many projections to the thalamus and cortex. We recently tested the role of mPFC projections in a nonspatial sequence memory task using a projection-specific DREADD approach in rats. We found that inhibiting mPFC projections to the nucleus reuniens of the thalamus (RE), or separately to perirhinal cortex (PER), produced opposing performance gradients across lag distance (Jayachandran et al. 2018). This suggests that each pathway differentially contributes to retrieval during sequence memory performance. Generally, the deficit patterns suggested that mPFC projections to RE contribute to a working memory strategy, whereas mPFC projections to PER contribute to a temporal context memory strategy. However, the question arose whether or not the same cells in mPFC project to both RE and PER, or whether these are separate non-overlapping cell populations. To address this question, we examined the mPFC->RE and mPFC->PER pathways using a dual retrograde fluorescence labeling experiment by injecting cholera toxin subunit-B green (CTB-488) and red (CTB-594) into RE and PER (unilaterally). We analyzed cells in the anterior cingulate (ACC), prelimbic (PL), and infralimbic (IL) cortices of the mPFC for retrograde labeling from either RE and PER, and examined cell densities across layers. mPFC->RE projecting cells were found in layers II, V, and VI, with the highest density in layer VI. mPFC->RE projecting cells were found throughout PL and IL, and only a few cells were found in ACC. mPFC->PER projecting cells were primarily observed throughout layers III and V, and most dense in PL, but were also found in IL. We did not find cells in mPFC that projected to both RE and PER (i.e., no dual-labeled cells were found). These results show that mPFC projections to RE and PER originate from two separate non-overlapping cell populations (RE: layers 2/3, 5, and 6) and (PER: layers 2/3 and 5). Speculatively, this cell/circuit segregation may provide mPFC with a simple mechanism that allows memory to be engaged with different retrieval strategies.

Disclosures: M. Schlecht: None. **M. Jayachandran:** None. **S.B. Linley:** None. **S.V. Mahler:** None. **R.P. Vertes:** None. **T.A. Allen:** None.

Poster

242. Learning and Memory: Cortical-Hippocampal Interactions I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 242.24/X3

Topic: H.01. Animal Cognition and Behavior

Title: Learning related changes in hippocampal and caudate activations for conditional associations

Authors: *A. G. HAMM[1], A. T. MATTFELD[2];
[1]Florida Internation Univ., Miami, FL; [2]Psychology, Florida Intl. Universit, Miami, FL

Abstract: Successful decision-making often relies on the ability of an individual to build and maintain conditional associations. For instance, choosing to select one option over another may be conditional on your prior experience or a change in context. In previous work, our findings demonstrated distinct neurobiological networks through which memory either prospectively biased decision-making or influenced the subsequent execution of behavior. The manner in which these mechanisms evolve over the course of learning, however, remains an important question. Here, we elucidate the neurobiological mechanisms related to changes in conditional behavior across learning period and strength. Opposite patterns of activations emerged for the hippocampus (HPC) and dorsal anterior caudate (DC) across learning: (1) increased learning-related activation in the HPC and (2) decreased activation in the dorsal DC was observed as memory strength increased. In addition, a parametric modulation analysis supported these findings, demonstrating significantly increased HPC, and decreased DC, activation associated with probability of correct response. Thus, the findings of our learning analysis demonstrate regions which were found to be involved in both prospective (HPC) and concurrent (DC) memory processes differentially contribute to the learning of conditional associations.

Disclosures: A.G. Hamm: None. **A.T. Mattfeld:** None.

Poster

242. Learning and Memory: Cortical-Hippocampal Interactions I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 242.25/X4

Topic: H.01. Animal Cognition and Behavior

Support: NS108259
MH113626

Title: Dissociable effects of the thalamic nucleus reuniens and ventromedial prefrontal cortex in executive functioning in the rat

Authors: *A. K. P. ROJAS[1], M. SCHREIBER[1], A. ATHANASON[1], T. A. ALLEN[2], S. B. LINLEY[1], R. P. VERTES[3];
[1]Florida Atlantic Univ., Boca Raton, FL; [2]Dept. of Psychology, Florida Intl. Univ., Miami, FL; [3]FAU/Ctr Complex Systems, Boca Raton, FL

Abstract: Executive functions are orchestrated by a diverse set of prefrontal (PFC) circuits that work both independently and in concert with one another to regulate cognition. While aminergic and frontostriatal networks are positioned at the forefront of behavioral control, the medial

thalamus has emerged as a key node in the executive network, presumably through its interconnections with the PFC. Of these, the nucleus reuniens (RE) of the ventral midline thalamus is pivotal in both mnemonic and cognitive processes and is strongly and reciprocally connected to the PFC, most heavily targeting the ventromedial PFC (vmPFC), including the prelimbic, infralimbic, and medial orbital cortices. In the present study, we compared the role of RE, the vmPFC, and vmPFC projections to RE in the attentional set shifting task (AST), a paradigm which tests three discrete measures of executive functioning. Long Evans male rats (n=24) were injected with the AAV9 hM4Di designer receptor exclusively activated by designer drugs (DREADD) in RE or bilaterally in vmPFC, followed by implantation of an indwelling cannula in RE. Following recovery and full expression of the virus, rats were tested on an olfactory AST. Briefly, the AST consists of seven stages which assess attentional set, attentional set shifting, and reversal learning (RL) using response contingencies to a particular odor or tactile exemplar. We found clozapine-n-oxide (CNO) inhibition of RE through intraperitoneal injection (CNO, 5mg/kg) or direct intracerebral infusions (0.75ul of 1mg/mL) produced significant impairments across reversal learning stages in comparison to vehicle infusions. Furthermore, impairments in RL were associated with significantly more perseverative errors in these stages, reflecting a failure to extinguish responding to the previous response contingency. By comparison, CNO inhibition of the vmPFC by intraperitoneal injection (5mg/kg) produced significant impairments in attentional set formation, but these rats were not impaired during reversal stages. Interestingly, inactivation of vmPFC projections to RE did not produce a significant effect of performance on the task, suggesting prefrontal input to RE does not regulate this aspect of the task. These results demonstrate that while RE and the vmPFC have dissociable roles in the AST task, inhibition of these structures disrupt flexible adaptive behavior. Additionally, these findings support the neural correlates of attentional deficits and cognitive inflexibility witnessed in CNS disorders including obsessive compulsive disorder, addiction, and schizophrenia, in which both frontal and thalamocortical dysregulation are present.

Disclosures: **A.K.P. Rojas:** None. **M. Schreiber:** None. **A. Athanason:** None. **T.A. Allen:** None. **S.B. Linley:** None. **R.P. Vertes:** None.

Poster

242. Learning and Memory: Cortical-Hippocampal Interactions I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 242.26/X5

Topic: H.01. Animal Cognition and Behavior

Support: NS108259
MH113626

Title: Chemogenetic inhibition of the nucleus reuniens of the thalamus mediates anxiety-like behavior in the rat

Authors: *A. ATHANASON[1], M. SCHREIBER[1], S. B. LINLEY[1], R. P. VERTES[2];
[1]Florida Atlantic Univ., Boca Raton, FL; [2]FAU/Ctr Complex Systems, Boca Raton, FL

Abstract: Hippocampal-prefrontal interplay is an integral aspect of cognition and behavior. While the hippocampus (HF) directly projects to the prefrontal cortex (PFC), PFC influences the HF through indirect routes. The nucleus reuniens (RE) of the ventral midline thalamus is the principal intermediary between the HF and PFC, and alterations of RE produce impairments in cognitive and mnemonic behaviors that require the cooperation of HF→ PFC circuitry. However, HF→ PFC synchrony is also involved in affective behavior and its dysregulation is prominent in psychiatric disease including schizophrenia, depression, and anxiety. In the rat, the ventral hippocampus (vHF) and medial prefrontal cortex (mPFC) are involved in anxiogenic-like behavior. Electrophysiological data indicates highly synchronized theta activity between the vHF and mPFC in anxiogenic tasks, supporting the importance of communication between these structures in anxiety. To test whether RE participates in this cortico-hippocampal circuit, the present study examined the effects of chemogenetic inhibition of RE and RE projections to the vHF and mPFC in anxiety like behavior using a one trial elevated plus maze (EPM) task. Long Evans male rats (n=8) were microinjected with the hM4Di designer receptors exclusively activated by designer drugs (DREADD) in RE, and indwelling cannula guides targeting RE, mPFC or bilaterally in vHF were implanted. Following a minimum of two weeks for full DREADD receptor expression, rats underwent an infusion of clozapine-N-oxide dihydrochloride (0.75ul of 1ug/uL) or vehicle (physiological saline) 30 minutes before being exposed to a five minute trial in the EPM. CNO inhibition of RE increased anxiety-like behavior in rats as measured by more time spent in the closed vs. open arms in comparison to vehicle controls. The results indicate that RE is involved in mediating anxiety-like behavior in the EPM in rats, highlighting RE as a node in both affective and cognitive behavior.

Disclosures: A. Athanason: None. **M. Schreiber:** None. **S.B. Linley:** None. **R.P. Vertes:** None.

Poster

242. Learning and Memory: Cortical-Hippocampal Interactions I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 242.27/X6

Topic: H.01. Animal Cognition and Behavior

Title: Valence specific discrimination/generalization bias evident with increasing hippocampal and amygdala-hippocampal maturity

Authors: *A. KIMBLER, D. L. MCMAKIN, A. T. MATTFELD;
Florida Intl. Univ., Miami, FL

Abstract: The hippocampus is a structure with a protracted developmental trajectory - extending well into adolescence and is known to play an important role in forming new memories. Different subfields of the hippocampus are thought to play distinct roles in an organism's ability to discriminate and generalize across events. Specifically, the dentate gyrus is thought to contribute to pattern separation, the process of making overlapping representations more dissimilar, while the CA3 is thought to participate in pattern completion, the process of reinstating past representations from partial input. These subfields mature at different rates, and a shift appears from preferentially completing patterns (generalizing) in youth to preferentially separating patterns (discriminating). A recent study found that a novel metric of hippocampal maturity, using partial least squares correlations analysis to compute an index of maturity based on subfield volume and subject age, supported this shift in bias from generalization towards discrimination. Our study aimed to extend these findings across different image valences. We examined how subjects from ages 9 to 14 years performed on a scene recognition task with positive, negative, and neutral images. Using the same volume-based maturity analysis as the previous study, we found that as hippocampal maturity increased so did their ability to discriminate neutral images which is consistent with the prior study and in line with the notion that the subfields important for pattern separation develop later. Conversely, generalization of negative images increased with hippocampal maturity, suggesting that negative images are differentially treated by this circuit. To expand upon these findings, we created a similar maturity score based on the connectivity between the amygdala and different hippocampal subfields identified through probabilistic tractography. Using this approach, we found that as amygdala-hippocampal connectivity maturity increased so did generalization for both negative and neutral images. In contrast, as amygdala-hippocampal connectivity maturity increased discrimination of negative images decreased while neutral images remained unaffected.

Disclosures: A. Kimbler: None. **D.L. McMakin:** None. **A.T. Mattfeld:** None.

Poster

242. Learning and Memory: Cortical-Hippocampal Interactions I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 242.28/X7

Topic: H.01. Animal Cognition and Behavior

Support: MH113626
NS108259

Title: Thalamocortical interactions in memory for elapsed time

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Abstract: Elapsed time memory, or memory for "how long ago" an event occurred, is a critical component of episodic memory. Elapsed time memory is thought to be useful for segregating different episodes in memory, or linking closely occurring events. Interactions between the hippocampus (HF) and prefrontal cortex (PFC) are central to episodic memory, including for temporal components. Specifically, the HF, through its interconnections with neocortical structures including the PFC, serves a critical role in recalling both the sequential order of events and elapsed time. While the ventral HF (vHF) exerts direct influence over the medial PFC (mPFC), return projections from the mPFC to the vHF arrive through indirect pathways. A key node in PFC à HF circuitry is the nucleus reuniens (RE) of the ventral midline thalamus, which strongly and reciprocally connects to both the mPFC and HF and is involved in mnemonic functions which recruit the mPFC and HF, including contextual fear conditioning and extinction, spatial working memory, and sequence memory. However, it is unknown whether RE participates in elapsed time. In the present study, we utilized a novel behavioral paradigm modified from Jacobs et al. (2013) to examine elapsed time memory using a chemogenetic approach to examine the role of REàHF and REàPFC circuitry. Long Evans male rats (n=10) were trained on response contingencies which paired a directional response (L or R) in a T-maze with an elapsed time interval of two different time scales. Once criterion was reached (70% accuracy), rats were microinjected with the virally-delivered hM4Di DREADD (AAV9.CAG.mCherry-2a-hM4dnrxn.WPRE.SV40) targeting RE. In the same surgery, indwelling cannula targeting the mPFC, vHF, or RE were implanted in rats, such that clozapine N-oxide (CNO), which acts at hM4Di DREADD to inhibit neuronal activity could be infused directly into RE or to RE projections to the HF or PFC. Following recovery and retraining, rats were tested in a repeated-measures design. CNO-induced inhibition of RE or RE terminals did not impair the ability to discriminate larger temporal differences (1 vs.12 min; 30 s vs. 6 min) compared to vehicle infusions. By comparison, RE projections to both the mPFC and vHF impaired performance when the temporal differences were smaller (8 vs 12 min). These results suggest that RE, through its thalamocortical interactions with limbic structures, participates in both spatial and temporal aspects of memory.

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Poster

242. Learning and Memory: Cortical-Hippocampal Interactions I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 242.29/X8

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant MH113626
NIH Grant ES006189

Title: Delta and theta dynamics in medial prefrontal cortex and the hippocampus of behaving rats

Authors: *T. D. VIENA[1], N. W. SCHULTHEISS[1], J. MCGLOTHAN DZIEDZIC[2], T. R. GUILARTE[2], T. A. ALLEN[1];
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Abstract: The medial prefrontal cortex (mPFC) and hippocampus (HC) are connected through direct and indirect pathways, including the nucleus reuniens of the thalamus (RE). Interactions between mPFC and HC are thought to be mediated by oscillatory synchrony bands supporting cognition and behavior. Likewise, abnormal synchrony might contribute to numerous neuropsychiatric disorders including schizophrenia and ADHD. For example, rodent models of schizophrenia show reductions in delta band (1-4Hz) and theta band (5-11Hz) coherence between mPFC and HC. While it is known that mPFC-HC field potentials synchronize in the theta band in various cognitive tasks, questions remain about the relationship between delta and theta rhythms during awake behavior. To begin to examine this issue, we recorded rats implanted with electrodes in mPFC and CA1 while they searched for food dropped randomly in an open field (120 x 120 cm). We then defined behavioral epochs (of at least 2 s) as running bouts (>10 cm/s minimum) or stationary pausing (<5 cm/s maximum), and we calculated delta and theta power, frequency, and mPFC-HC coherence. We found that both delta and theta bands have prominent power in both mPFC and HC, but tended to be higher in HC. Delta and theta power were largely orthogonal in both structures and corresponded to behavior such that (1) higher delta power and delta-band mPFC-HC coherence were associated with stationary periods, and (2) higher theta power and theta-band mPFC-HC coherence were associated with active exploration. Theta- and delta-dominated epochs often transitioned rapidly (~1-2 s) coupled to shifts between behaviors. We've begun to examine the hypothesis that the orchestration of different oscillatory states in the mPFC-HC system is performed, in part, by RE activity (Doelleman-van der Weel et al., 2019). RE is reciprocally connected to both mPFC and HC (CA1 and subiculum), and populations of RE projection neurons exert powerful excitatory actions on either or both structures. We hypothesize that RE activity coordinates features of the observed mPFC-HC oscillatory states (e.g., transitions, frequency, power, and/or coherence).

Thus, in a separate group of rats, we've employed an AAV-retrograde strategy to express channelrhodopsin in RE cells that project to mPFC, HC, or both. We implanted electrodes in mPFC and HC, and an optrode in RE. This new approach enables stimulation of different RE populations to test the hypothesis that RE coordinates delta and theta states within the circuit. This ongoing work suggests different roles for delta and theta in awake behavior, likely coordinating functional interactions of the mPFC-RE-HC system.

Disclosures: T.D. Viena: None. **N.W. Schultheiss:** None. **J. McGlothan Dziedzic:** None. **T.R. Guilarte:** None. **T.A. Allen:** None.

Poster

243. Memory Engrams

Location: Hall A

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Topic: H.01. Animal Cognition and Behavior

Support: NIH DP5 Early Independence Award
McKnight Memory and Cognitive Disorders Award
NARSAD Brain and Behavior Research Foundation Award
Ludwig Family Foundation Grant
MH052090

Title: Hippocampus and amygdala fear memory engrams re-emerge after contextual fear reinstatement

Authors: *W. MAU[1], Y. ZAKI[5], C. R. CINCOTTA[2], O. P. MCKISSICK[6], M. SHPOKAYTE[3], A. HAMIDI[7], E. DOUCETTE[2], S. L. GRELLA[4], N. J. MURAWSKI[8], E. MERFELD[2], S. RAMIREZ[1];
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Abstract: The formation and extinction of fear memories represent two forms of learning that each engage the hippocampus and amygdala. How cell populations in these areas contribute to fear relapse, however, remains unclear. Here, we demonstrate that, in mice, cells active during fear conditioning in the dentate gyrus of hippocampus and basolateral amygdala exhibit decreased activity during extinction and are re-engaged after fear reinstatement. In vivo calcium imaging reveals that reinstatement drives population dynamics in the basolateral amygdala to revert to a network state similar to the state present during fear conditioning. Finally, we find that optogenetic inactivation of neuronal ensembles active during fear conditioning in either the

dentate gyrus or amygdala is sufficient to disrupt fear expression after reinstatement. These results suggest that fear reinstatement triggers a partial re-emergence of the original fear memory representation, providing new insight into the neural substrates of fear relapse.

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Poster

243. Memory Engrams

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 243.02/X10

Topic: H.01. Animal Cognition and Behavior

Support: NIH DP5 Early Independence Award
McKnight Memory and Cognitive Disorders Award
NARSAD Brain and Behavior Research Foundation Award
Ludwig Family Foundation Grant

Title: Population and projection-specific segregation of fear and reward engrams

Authors: ***M. SHPOKAYTE**[1], O. P. MCKISSICK[1], E. RUESCH[1], E. KRAMER[2], S. LIU[3], K. E. KITKO[4], S. L. GRELLA[1], E. DOUCETTE[1], E. MERFELD[1], S. RAMIREZ[1];
[1]Boston Univ., Boston, MA; [2]Hosp. For Sick Children, Toronto, ON, Canada; [3]Whitehead Inst. for Biomed. Res., Cambridge, MA; [4]Vanderbilt Univ., Nashville, TN

Abstract: The hippocampus is involved in a variety of mnemonic computations, including processing spatial-temporal dimensions of memory, as well as regulating stress-responses and processing emotional stimuli. Recent studies have demonstrated vast structural and functional heterogeneity along the dorsal-ventral axis of the hippocampus, and while much is known about how the dorsal hippocampus processes spatial-temporal content, much less is known about whether or not the ventral hippocampus (vHPC) contains defined populations and circuits capable of parsing out discrete emotional experiences. We find that optogenetic manipulation of tagged cell bodies in vHPC is not sufficient to drive fear or reward-related behaviors; however, optical activation of tagged vHPC terminals projecting to the amygdala and nucleus accumbens, but not the prefrontal cortex, are sufficient to drive preference and aversion, as well as to "switch" or "reset" their capacity to drive either. Subsequent RNA-sequencing analyses also revealed distinct genetic topographies for fear and reward-processing vHPC cells. Further, we combined two activity-dependent tagging strategies to develop a novel dual memory tagging scheme which, when combined with whole brain clearing, provide evidence that the vHPC recruits two partially segregated populations in response to rewarding or aversive stimuli. We

finally successfully tagged and manipulated two discrete memory engrams within the same animal in a variety of brain regions and projections, including the hippocampus and amygdala, to drive preference and aversion in a within-subject manner. Together, our findings suggest that separable monosynaptic vHPC outputs are functionally malleable and point to their genetic landscape as unique targets for potentially intervening with neurodegenerative diseases. Our ongoing experiments are focused on within animal optogenetic dual memory manipulation and brain wide tracing of fear and reward memory ensembles.

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Poster

243. Memory Engrams

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Topic: H.01. Animal Cognition and Behavior

Support: NIH DP5 Early Independence Award
McKnight Memory and Cognitive Disorders Award
NARSAD Brain and Behavior Research Foundation Award
Ludwig Family Foundation Grant

Title: Optogenetic induced extinction-like behaviors in ethanol withdrawn mice

Authors: *C. R. CINCOTTA[1], N. J. MURAWSKI[4], S. L. GRELLA[2], O. P. MCKISSICK[5], E. DOUCETTE[3], S. RAMIREZ[1];
[2]Ctr. for Memory and Brain, [3]Psychological and Brain Sci., [1]Boston Univ., Boston, MA; [4]Ctr. for Brain Sci., Harvard Univ., Cambridge, MA; [5]Northeastern Univ., Boston, MA

Abstract: Withdrawal from chronic alcohol impacts the brain's stress and memory systems, which may underlie individual susceptibility to persistent drug seeking and stress-induced relapse. Preclinical studies demonstrate impaired fear memory processes in rodents withdrawn from alcohol, including abnormally heightened fear responses that are resilient to subsequent attenuation by extinction training. The underlying neural circuits mediating, and sufficient to intervene with, impaired extinction following alcohol withdrawal have remained elusive. First, we demonstrate that mice withdrawn from chronic ethanol show impaired fear extinction and heightened fear renewal relative to controls. We next labeled neural ensembles in the dentate gyrus (DG) with an inducible and activity-dependent virus cocktail which allows expression of channelrhodopsin-2 (ChR2) or a control protein (eYFP) in cells active during the formation of a contextual fear memory in mice withdrawn from chronic alcohol. Mice were placed into a

distinct context and received chronic light stimulation of DG cells processing a fear memory over five consecutive days. Chronic reactivation of this fear ensembles led to context-specific reductions in fear responses in control mice expressing ChR2 (but not eYFP), suggesting that our stimulation strategy was successful in producing extinction-like behavior effects in ethanol withdrawn mice. These results offer insight into how chronic reactivation of fear ensembles in the hippocampus offer a means to facilitate extinction following withdrawal from chronic alcohol exposure. A mechanistic understanding of fear processes following drug withdrawal will aid in the development of therapies to attenuate stress-related cognitive dysfunction following drug withdrawal.

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Poster

243. Memory Engrams

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Topic: H.01. Animal Cognition and Behavior

Support: NSF NRT UtB: Neurophotonics
NIH DP5 Early Independence Award
McKnight Memory and Cognitive Disorders Award
NARSAD Brain and Behavior Research Foundation Award
Ludwig Family Foundation Grant

Title: Differentially driving defensive-like behaviors with the same activated fear engram

Authors: *K. DORST[1], O. P. MCKISSICK[4], J. H. BLADON[2], S. RAMIREZ[3];
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Abstract: This research aims to provide a framework for the network interactions that generate differential defensive responses to aversive stimuli. Specifically, the neural circuitry that mitigates various defensive behaviors associated with post-traumatic stress disorder (PTSD) and anxiety as a result of aberrant memory recall are largely unknown, and these behaviors manifest as distinctive outputs depending on both the brain state and the environment of the subject. For instance, a battery of common defensive behaviors that are symptomatic of both PTSD and anxiety include, but are not limited to: active avoidance, exaggerated startle response, and freezing. However, the neural circuitry sufficient to mitigate such defensive behaviors mediated by the recall of a specific fear memory is unknown. Here, we alter environmental contingencies to test for the capacity of a defined set of either hippocampal or basolateral amygdala cells to

differentially drive defensive behaviors when optogenetically activated. Our preliminary results show that artificial reactivation of the same set of cells processing fear manifests as increased running, anxiogenic responses, or freezing behavior, depending on whether these cells are stimulated during running, in an open field, or in a small chamber, respectively. These results suggest that a subset of cells, upon activation, recruit different neural substrates to generate diverging behavioral outputs. Our current work utilizes immunohistochemical analyses to identify candidate regions mediating state-dependent defensive behavioral switches. Additionally, we are testing for potential interactions between artificially reactivating a fear engram during bouts of running and subsequent fear memory recall, as aerobic exercise has been shown to augment memory retention. Together, our work provides insight into the capacity of discrete sets of cells to produce varying behavioral responses.

Disclosures: **K. Dorst:** None. **O.P. McKissick:** None. **J.H. Bladon:** None. **S. Ramirez:** None.

Poster

243. Memory Engrams

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Topic: H.01. Animal Cognition and Behavior

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McKnight Memory and Cognitive Disorders Award
NARSAD Brain and Behavior Research Foundation Award
Ludwig Family Foundation Grant

Title: Social and affective events modulate reactivation of memory ensembles

Authors: *A. B. FINKELSTEIN[1], H. LEBLANC[1], T. GALLERANI[1], R. COLE[1], A. HAMIDI[2], Y. ZAKI[3], S. RAMIREZ[1];
[1]Boston Univ., Boston, MA; [2]Broad Inst., Cambridge, MA; [3]Grad. Sch. for Biomed. Sci., Mount Sinai Icahn Sch. of Med., New York, NY

Abstract: The retrieval of memories is at the core of adaptive behavioral responses. Neuroscience has made great strides in understanding mechanisms of memory retrieval, but thus far tends to study these processes in neutral conditions isolated from other salient experiences. For animals in the natural world, decisions guided by prior learning are biased by recent experience. Novel behavioral paradigms combined with tools to manipulate defined sets of cells allow us to untangle the mechanisms that determine which memories an animal can access at any given moment. We show that auditory and chemical cues from a stressed cage-mate induce reinstatement of an extinguished fear memory, and reveal that this reinstatement necessitates reactivation of the basolateral amygdala fear memory ensemble. In addition to the impact of such

social experiences, we also show that recent affective experiences of positive vs negative valence give rise to suppressed vs enhanced fear memory recall, respectively, and modify the rate of extinction. The reactivation of associated fear memory ensembles diverges in the basolateral amygdala but not dentate gyrus of the hippocampus. Our findings reveal the capacity of an animal's current state to govern which memories are used to guide behavior.

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Poster

243. Memory Engrams

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Topic: H.01. Animal Cognition and Behavior

Support: NIH DP5 Early Independence Award
McKnight Memory and Cognitive Disorders Award
NARSAD Brain and Behavior Research Foundation Award
Ludwig Family Foundation Grant

Title: Perturbation of dorsal hippocampal ensembles disrupts memory stability

Authors: *S. L. GRELLA[1], J. H. BLADON[1], A. H. FORTIN[1], Y. ZAKI[4], C. R. CINCOTTA[1], O. P. MCKISSICK[5], M. SHPOKAYTE[2], E. DOUCETTE[1], E. MERFELD[1], S. RAMIREZ[3]; [1]Psychological and Brain Sci., [2]Dept. of Psychological Brain Sci., [3]Boston Univ., Boston, MA; [4]Grad. Sch. for Biomed. Sci., Mount Sinai Icahn Sch. of Med., New York, NY; [5]Northeastern Univ., Boston, MA

Abstract: A variety of intracranial self-stimulation (ICSS) paradigms have been used to investigate reward processing. Previously, we used an activity-dependent and doxycycline-controlled, inducible neuronal tagging system (virus cocktail of c-Fos-tTA & TRE-ChR2-eYFP) combined with optogenetics, to tag cells involved in processing a positive memory in mice (i.e. male and female social interaction). Here, we labeled these cells active during encoding in the dorsal dentate gyrus (dDG). Mice were then fear conditioned and during recall 24 hrs later, we optically reactivated this memory to disrupt memory stabilization processes and reduce subsequent fear responses. Our manipulation worked most effectively when we used a positive (rather than neutral or negative) experience and when stimulation was delivered in the first half of the session.
Next, we attempted to enhance the valence of the tagged positive memory using a viral injection of CAMKIIa-ChR2-eYFP targeted at the ventral tegmental area (VTA), where dopamine (DA), a neurotransmitter heavily implicated in reward processing is synthesized. We trained animals to

nose-poke for optical stimulation of the VTA which induces DA release similar to ICSS. Subsequently, the dDG cells active during VTA self-stimulation were tagged. We assessed: 1) whether animals would nose poke for dDG stimulation (i.e. memory of the VTA stimulation experience and 2) whether artificial reactivation of this positive memory would be more effective in disrupting fear memory stabilization following recall. This manipulation resulted in a significant reduction in fear compared to our first experiment.
Finally, we investigated whether non-specific activation of a random set of dDG cells would modulate subsequent fear responses by injecting a diluted CAMKIIa-ChR2-eYFP virus into the dDG. We found that activating cells indiscriminately compared to activating a subset of cells processing a discrete memory was also an effective method to modulate fear.
These results demonstrate that perturbation of dDG ensembles can affect memory stability, as has been seen in the past with electro-convulsive shock, and that disruption of a fear memory can be achieved using highly salient and positive memories.

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Poster

243. Memory Engrams

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Early Independence Award: DP5 OD023106-01
NIH R01 MH052090
NIH R01 MH051570

Title: Imaging the hippocampus to investigate the specificity and stability of long-term contextual fear memories

Authors: *N. R. KINSKY, *D. J. ORLIN, E. A. RUESCH, S. RAMIREZ;
Boston Univ., Boston, MA

Abstract: Despite the ubiquitous use of contextual fear conditioning (CFC) to study episodic memory, there is a knowledge gap pertaining to how the neural code evolves through the consolidation of short term memory into long-term memory. Previous studies (Moita et al., 2010; Wang et al., 2012) have demonstrated that CFC causes a reorganization, or remapping, of hippocampal place fields suggesting that the hippocampal neural code might provide a substrate for the retention and recall of CFC memories memory retention. To build upon these studies, we leveraged the spatial and temporal resolution of single-photon calcium imaging in conjunction

with CFC, in freely moving mice, to further characterize the heterogeneity of hippocampal remapping over long-time scales. Mice were exposed to two different arenas throughout the experiment, which allowed us to test the specificity of the CFC memory. Moreover, we tracked behavior in the days before, during, and after shock to examine the long-term stability of the CFC memory. One cohort of mice received anisomycin - a protein synthesis inhibitor that disrupts long-lasting synaptic plasticity - immediately after shock to block consolidation. We hypothesized that anisomycin would temporarily arrest learning-related changes in the event rate of neurons and turnover of active neurons between sessions, and would prevent remapping of their place field locations. We found that freezing returned to pre-shock levels in the days following CFC for anisomycin mice but not for control mice corroborating previous findings. Surprisingly, we also observed that anisomycin temporarily accelerated the turnover of active neurons. This increased cell turnover was concomitant with a general shutdown of neural activity; i.e. a drop in the number of active neurons we detected as well as a decrease in their event rate four hours to two days later. The amnestic effects of anisomycin could thus stem from the disruption of constitutive processes in conjunction with blocking learning-related plasticity. Additionally, the degree to which mice discriminated between arenas - as reflected by freezing more in the CFC chamber - correlated with the amount of cell turnover between the arenas. This finding supports the idea that the hippocampal neural code provides a substrate for differentiating between arenas. Future analyses will investigate the stability of learning-induced place field remapping during successful versus failed memory consolidation.

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Poster

243. Memory Engrams

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IMHRO/OneMind

Title: Mapping single neuron projections and input-output circuitry of the ventral hippocampus

Authors: *M. M. GERGUES[1], K. HAN[2], B. BROWN[3], K. CLAUSING[2], H. CHOI[2], V. S. TURNER[1], M. KHEIRBEK[2];
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Abstract: The ventral hippocampus (vHPC) and its extended circuitry have been widely implicated in various behavior states associated with reward processing, fear memory, anxiety, and other emotional states. This is due to the neuroanatomical organization of the vHPC as it sends projections to various limbic structures such as, prefrontal cortex (PFC), nucleus accumbens (NAc), lateral hypothalamus (LH), bed nucleus of the stria terminalis (BNST), lateral septum (LS) and basal amygdala (BA), all areas which have been independently shown to regulate different features of affective behavior. Recent work from our group has shown that these outputs are functionally dissociable, vHPC-BA outputs are involved in the encoding of context fear, while vHPC-LH projections are important for anxiety-related behavior. However, the anatomical organization of vHPC outputs, with respect to their inputs and outputs, and the molecular identifiers that may dissociate between output pathways remains unknown. Here, we used viral and sequencing approaches to address these questions to map out the vHPC circuit. Using Map-Seq, a high-throughput method for mapping the connectivity of single neurons, we mapped the projection patterns of hundreds of vHPC neurons and found that while a large proportion of cells send outputs to single downstream targets, a number of multi target motifs were overrepresented in our dataset. Next we used a rabies virus input-output tracing approach to determine the differential inputs of vHPC cells projecting to either the PFC, NAc, BA, and LH. Finally, current efforts are focused on assessing the molecular profile of these output neurons using a translating ribosome affinity purification approach. These studies highlight the rich heterogeneity of vHPC neurons and provide novel targets for future interventions for the regulation of components of affective behavior.

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Poster

243. Memory Engrams

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R01 MH117961
Weill Scholar Award
IMHRO/One Mind Rising Star Award
NARSAD Young Investigator Grant

Title: Encoding of innate and learned stimuli in the ventral hippocampus

Authors: *J. BIANE[1], M. A. LADOW[1], F. STEFANINI[2], S. P. BODDU[1], D. L. APODACA-MONTANO[1], A. FAN[1], N. I. WOODS[1], S. CHIEN[1], M. KHEIRBEK[1];
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Abstract: Cues in the environment can have innate valence to animals, or can gain salience via associative learning. Recent reports have indicated that in a number of brain areas cues of differing valences, or differing modalities of the same valence, may be processed by distinct ensembles of neurons. Studies in the ventral hippocampus have suggested that anatomically separable populations of neurons may differentially encode stimuli of distinct valence, however the degree to which these representations are stable across time, stimulus modality, or valence identity remains unclear. To address these questions, we tracked real-time activity of individual neurons in the ventral hippocampus (vHPC) using 2-photon imaging while head-fixed mice were exposed to a battery of stimuli that included positive (sucrose, appetitive odor) and negative (shock, aversive odor) valence across multiple days. Within vCA1, neural representations showed minimal similarity across stimuli, including for stimuli with similar valence (eg, shock and aversive odor), suggesting that vCA1 representations are biased toward stimulus identity and not valence class *per se*. Across days, neural responses to the same stimulus were moderately stable, depending on the stimulus class. Shock responsive cells were most stable across days, with less stability seen for odor and sucrose responsive neurons. To determine whether neurons responding to unconditioned stimuli (US) begin to respond to cues (CS) that predict the US through associative learning, a separate cohort of mice underwent tone-sucrose trace conditioning during 2-photon imaging of vCA1, and neurons were tracked across imaging sessions. Whereas the neural representations to the US again showed moderate stability over days, the representation of the predictive tone cue was dynamically transformed with learning, with CS-responsive neurons emerging over the course of training. However, unlike previous reports in amygdala, CS and US representations showed little evidence of convergence following learning, further suggesting that vCA1 maintains distinct representations of discrete stimuli, regardless of valence similarity or predictive relationship between stimuli. Ongoing experiments are exploring vCA1 representations across a greater diversity of valent stimuli, both learned and innate.

Disclosures: J. Biane: None. **M.A. Ladow:** None. **F. Stefanini:** None. **S.P. Boddu:** None. **D.L. Apodaca-Montano:** None. **A. Fan:** None. **N.I. Woods:** None. **S. Chien:** None. **M. Kheirbek:** None.

Poster

243. Memory Engrams

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 243.10/X18

Topic: H.01. Animal Cognition and Behavior

Support: Canadian Institutes of Health Research
Natural Sciences and Engineering Research Council of Canada
Canadian Institute For Advanced Research
Brain & Behavior Research Foundation
Ontario Student Assistance Program
Hospital for Sick Children Restracomp Fellowship

Title: Visualizing the basolateral amygdala engram

Authors: *E. E. KRAMER, P. E. STEADMAN, A. D. JACOB, A. PARK, P. W. FRANKLAND, S. A. JOSSELYN;
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Abstract: An engram or memory trace is the neural substrate representing a past experience that can drive future behaviour. While engrams may be localized across many regions of the brain, the amygdala in particular is known to be critical for the encoding and storage of associative memories. However, the mechanisms that determine which amygdala neurons are recruited to an engram are not well known. Our goal is to understand which neurons are active during memory encoding or recall throughout the whole basolateral amygdala. Using the spatial positions of active neurons, we can determine how different subnuclei of the amygdala are recruited based on the stage of memory formation (encoding or recall), or the valence (aversive vs appetitive experience). Wild-type mice (8-10 weeks) were trained with either aversive cued fear conditioning (tone), aversive context fear conditioning, appetitive conditioned place preference pairing with cocaine or saline, or remained in home cage (n=6 each group). Mice were perfused 90 min after training or 90 min following testing the next day. Brains were fixed, sectioned, and cleared using a modified iDISCO+ protocol. Nuclei were stained using SYTOX orange and Arc protein was detected using immunohistochemistry, as a marker of active neurons. Samples were imaged using a LaVision Ultramicroscope light sheet microscope and Imspector software. Mice trained with a tone-shock pairing showed greater total Arc expression in the lateral amygdala (LA, ~10%) compared to the basal amygdala (BA, ~6%). Mice trained in contextual fear conditioning showed high Arc expression in both the LA (~9%) and BA (~11%). The position of Arc+ cells was analyzed and compared to all nuclei positions to examine spatial clustering. Overall, mice in encoding or recall conditions show a spatial pattern of Arc expression with greater local clustering than home cage mice. No consistent large-scale clustering of Arc+ cells was detected along an anterior-posterior, dorsal-ventral, or medial-lateral axis within behavioural groups. These clustering results suggest that neurons that are active during encoding or recall are distributed throughout both the LA and BA. However, the presence of local clustering suggests that those neurons which are active during encoding or recall are not randomly spatially recruited. These spatial analyses suggest that neurons are activated in relatively small groups when animals encode and recall salient experiences. Further analysis will examine specific spatial sub-regions of amygdala subnuclei to identify any consistencies in the location of these small clusters of active neurons across animals.

Disclosures: E.E. Kramer: None. **P.E. Steadman:** None. **A.D. Jacob:** None. **A. Park:** None. **P.W. Frankland:** None. **S.A. Josselyn:** None.

Poster

243. Memory Engrams

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 243.11/X19

Topic: H.01. Animal Cognition and Behavior

Support: CIHR Foundation Grant FDN143227

Title: Whole brain mapping of networks engaged during encoding and retrieval of contextual fear memory

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Abstract: Memory retrieval is thought to re-engage neural ensembles that were active during initial encoding. Support for this view has emerged from studies genetic tagging strategies that allow labeling of active neuronal ensembles in a temporally defined and inducible manner (e.g., TetTag and TRAP lines). However, these analyses have, for the most part, been limited to one or only a limited number of brain regions of interest. Here we address this question using whole brain analysis of active neuronal ensembles during encoding and retrieval of a contextual fear memory in mice. To do this, Fos-TRAP mice (Guenthener et al, 2013) were trained in contextual fear conditioning and tested 2 days later. Brains were cleared using iDISCO and immunolabeled for TdTomato (neurons active during encoding) and c-Fos (neurons active during retrieval). Brains were imaged using light-sheet microscopy. Next, we processed the images with a pipeline consisting of stitching image tiles (ImageJ), automated cell counting, registration and segmentation to the Allen Brain Atlas reference (ClearMap; Ranier et al, 2016). In order to identify regions co-activated during memory encoding or retrieval, we next computed a complete set of inter-regional correlations. Low ($P<0.01$), medium ($P<0.005$) and high ($P<0.0025$) confidence networks were constructed from these correlations, and both local and global network metrics calculated for resulting graphs. Within these networks we identified hubs common to both encoding and retrieval (e.g., temporal association, ectorhinal and olfactory cortices) and hubs that were prominent in either only in the encoding (e.g., diagonal band nucleus, prelimibic and orbital cortices) or the retrieval (e.g., retrosplenial and anterior cingulate cortices) network. Moreover, we computed the community structure of the encoding and retrieval networks. The biggest 3-4 communities of both networks had very similar composition. In addition, regions that bridge communities (i.e., regions with high participation coefficients) were computed. Many of these were common to both networks (e.g., dentage gyrus, central amygdala, ectorhinal and

dorsal retrosplenial cortices). However, some bridging regions were more prominent for encoding (e.g., anterior basal amygdala and prelimbic cortex) or retrieval (CA3, perirhinal cortex and anteromedial thalamic nucleus for retrieval). These analyses generate data-driven hypotheses about the roles of individual brain regions in encoding/retrieval networks that can be evaluated using causal neuroscience interventions (e.g., optogenetic activation and or silencing).

Disclosures: C.A. Coelho: None. **S.A. Josselyn:** None. **P.W. Frankland:** None.

Poster

243. Memory Engrams

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Topic: H.01. Animal Cognition and Behavior

Support: CIHR
NSERC PGSD
Vector Institute PGA
SickKids Restracomp

Title: Computational evidence for the role of neurogenesis in memory generalization

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Abstract: The dentate gyrus of the hippocampus is one of two neurogenic niches in the adult mammalian brain, where newborn neurons are constantly integrated into the local circuitry throughout life. These immature neurons (3-4 weeks of age) in the dentate gyrus are hyperexcitable, have fewer inhibitory inputs, and are more plastic than their mature counterparts. We predict that this unique combination of physiological properties in immature neurons of the dentate gyrus, a region known for its sparse firing, may contribute noise to neural activity during learning. It is well known in machine learning that noise injection is one way to prevent overfitting in artificial neural networks (ANNs) and promote generalization, that is the ability to extract statistical regularities from previously encountered data in order to make predictions about new, previously unseen data. Therefore here we hypothesize that neurogenesis is one such source of noise in the hippocampus, and may thus promote memory generalization. To test this hypothesis, we implemented neurogenesis in different ANN architectures trained to classify images from the MNIST handwritten digits or CIFAR10 image datasets. We find that not only does neurogenesis enhance generalization, neurogenic networks are also more robust to neuronal

ablation indicating less reliance on individual units and more on the population of activations instead. To test whether neurogenesis is also important for generalization in behaving mice, we are developing a visual categorization touchscreen task to compare generalization performance in irradiated mice (with no neurogenesis) and non-irradiated mice (intact neurogenesis). In this task, must learn to categorize a set of exemplar images drawn from the MNIST dataset during the training phase, and we assess generalization by measuring correct categorization of novel MNIST exemplars in the probe phase. The results of this work can provide insight into the link between neurogenesis and the mechanisms underlying generalization in the hippocampus.

Disclosures: L.M. Tran: None. **A. Santoro:** None. **S.A. Josselyn:** None. **B.A. Richards:** None. **P.W. Frankland:** None.

Poster

243. Memory Engrams

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 243.13/X21

Topic: H.01. Animal Cognition and Behavior

Support: NSERC
CIHR

Title: Imaging neuronal allocation to an episodic-like memory in the rodent hippocampus

Authors: *A. J. MOCLE[1], A. I. RAMSARAN[2], B. A. RICHARDS[3], P. W. FRANKLAND[4], S. A. JOSSELYN[5];
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Abstract: Episodic-like memories are thought to be encoded by the activity of a sparse ensemble of neurons in the hippocampus of rodents, termed its memory engram. Recent work characterizing engrams of different types of memories have pointed to a common excitability-dependent mechanism of neuronal allocation to an engram. Neurons that are highly excitable relative to their neighbours will be preferentially recruited to encode a memory. This enhanced excitability is typically produced by manipulating a sparse population of neurons either by genetic or optogenetic methods. Furthermore, it is thought that recall of a previously acquired memory transiently enhances the excitability of its engram, thereby allowing its modification, updating, or linking with new memories.
Despite strong evidence for an excitability-dependent mechanism for neuronal allocation, it primarily comes from studies involving the experimental manipulation of neuronal excitability. It is possible that these manipulations push neuronal excitability beyond of the normal biological

range, and may therefore not recapitulate endogenous mechanisms. In this study, we take advantage of a custom miniaturized microscope to investigate endogenous mechanisms of neuronal allocation. Our data suggests that highly excitable principal neurons in CA1 are more likely to be allocated to a hippocampal memory trace. Furthermore, we show that the excitability-dependent competition likely occurs between small ensembles of neurons that are present immediately before training, and which are reactivated to support recall.

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Poster

243. Memory Engrams

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Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 243.14/X22

Topic: H.01. Animal Cognition and Behavior

Support: CIHR
NSERC

Title: Neurogenesis impairs fear expression and alters CA1 population dynamics during memory recall

Authors: ***A. I. RAMSARAN**[1], A. J. MOCLE[2], L. M. TRAN[2], A. D. JACOB[3], J. C. JIMENEZ[4], M. KHEIRBEK[5], S. A. JOSSELYN[6], P. W. FRANKLAND[7];
[1]The Hosp. for Sick Children, Toronto, ON, Canada; [3]Psychology, [2]Univ. of Toronto, Toronto, ON, Canada; [4]Columbia Univ., New York, NY; [5]Univ. of California, San Francisco, San Francisco, CA; [6]Neurosci. & Mental Hlth., [7]PGCRL - NMH 5th floor, Hosp. for Sick Children, Toronto, ON, Canada

Abstract: In most mammals, new neurons are born in the dentate gyrus (DG) throughout life. The subsequent integration of these new neurons into the hippocampus effectively reorganizes the local circuitry in which episodic memories are stored, suggesting a role for neurogenesis in weakening previously-encoded memories. Consistent with this idea, our lab has shown in silico (using an artificial neural network) and in vivo (using interventions like voluntary running in mice) that high rates of neurogenesis following memory formation causes forgetting of hippocampus-dependent memories. What remains poorly understood is how the integration of newly-generated neurons into DG-CA3 circuits impairs the retrieval of memory representations in downstream brain regions. To address this question, in the current study we used custom miniaturized endoscopes to record calcium activity from freely-behaving transgenic mice expressing GCaMP6f in subfield CA1 of the hippocampus. We monitored the activity of thousands of CA1 pyramidal neurons while mice formed a contextual fear memory and recalled

this memory during two test sessions. Memory was first assessed in a recent test one day after training during which all mice successfully recalled the fear memory, and after the recent test we provided running wheels to a subset of mice for one month to promote neurogenesis. Similar to our previous studies, mice that ran showed robust forgetting compared to controls during the remote test. We analyzed single-cell data from each session and observed that neurogenesis did not alter the number of neurons active during the remote test or the number of neurons from previous sessions reactivated during the remote test. Using machine learning classifiers, we further found that behavioral states (freezing versus non-freezing) could be reliably decoded from neuronal activity in all mice during the recent test. However, freezing classification was diminished in mice with elevated neurogenesis during the remote test, suggesting that CA1 population dynamics poorly represent memory-related states following neurogenesis-mediated forgetting. These preliminary results reveal the functional consequence of hippocampal neurogenesis on memory-related CA1 population activity and begin to address the neural circuit mechanism by which forgetting occurs in the brain.

Disclosures: A.I. Ramsaran: None. **A.J. Mocle:** None. **L.M. Tran:** None. **A.D. Jacob:** None. **J.C. Jimenez:** None. **M. Kheirbek:** None. **S.A. Josselyn:** None. **P.W. Frankland:** None.

Poster

243. Memory Engrams

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Topic: H.01. Animal Cognition and Behavior

Support: NRF 2018R1A2B3004486

Title: Potentiated synapses are included into memory engram through a two-step mechanism

Authors: *Y. JEONG, H.-Y. CHO, M. KIM, J.-P. OH, M. KANG, M. YOO, H.-S. LEE, J.-H. HAN;
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Abstract: Memory is thought to be represented by learning-induced physical changes in the brain, referred as an engram. Synapse is a brain location where such long-term changes occur. Evidence supports that memory trace exists at potentiated synapses. However, how synapses are allocated into memory engram after learning is not well understood. Here, in auditory input to amygdala synaptic connections, we used optogenetic input stimulation prior to fear conditioning to tag weak inputs normally not potentiated (opto-tagging). Such tagging stimulation led to formation of long-term potentiation (LTP) at the tagged synapses in a learning-dependent manner. Reactivation of the tagged synapses both sufficiently elicited freezing and rendered tone fear memory labile as in reconsolidation. On the contrary, photoinhibition and depotentiation of

the tagged synapses, during retention and 6 h after conditioning, respectively, blocked natural tone fear memory recall. However, canceling of potentiation on the tagged synapses right after the training by LTD did not attenuate the memory and showed that those synapses failed to be allocated into fear memory engram in this case. These results collectively reveal that potentiated synapses are allocated into fear memory engram through two distinct steps, supporting a tagging and capture-like model as a plausible mechanism for synaptic memory allocation.

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Poster

243. Memory Engrams

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Topic: H.01. Animal Cognition and Behavior

Support: Samsung SSTF-BA1801-10

Title: Inactivation of old engram cells during memory update by retraining

Authors: *H.-Y. CHO, H.-S. LEE, J. HAN, Y. LEE, J.-H. HAN;
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Abstract: Repetition of an experience results in updating of the memory. It has never been clearly addressed how such update is represented at the memory engram level. Reconsolidation theory posits that re-exposure to an event reactivates the acquired trace, transiently making it labile, allowing further modifications to the trace. On the other hand, many human studies report the importance of “forgetting” or inhibition of the old memory for successful memory updating. What then is the fate of the old memory trace when repeated experience updates the memory? Using both artificial memory allocation and natural engram tagging methods, we found that the original memory trace is no longer necessary for the recall of retrained fear memory. Such result was accompanied by a decrease in the reactivation probability of initially allocated engram cells during retrieval of retraining memory. We also found that although the initial memory engram loses its necessity for recall of the fear memory after repeated training, these neurons still retain the fear memory as direct activation of the same neurons was sufficient for inducing a fear response. Therefore, our results demonstrate inactivation of old engram cells in the amygdala during fear memory update after retraining and suggest creation of a new memory trace as a possible mechanism for memory update at the engram level.

Disclosures: H. Cho: None. **H. Lee:** None. **J. Han:** None. **Y. Lee:** None. **J. Han:** None.

Poster

243. Memory Engrams

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Title: Nmda receptor is required for systems consolidation and sequential printing of fear memory engrams

Authors: *M. T. HASAN[1,2,3,4], I. BERTOCCHI[2,5], G. K. DOGBEVIA[2], F. ROCHA-ALMEIDA[6], M. TREVINO VILLEGAS[2,7], P. BOTTA[8,9], P. MELE[5], J.-M. WEISLOGEL[10], E. TUNKL[2], M. ROβMANITH[2], D. ARCOS-DIAZ[2], M. CAMBIAGHI[11], A. CARRETERO-GUILLEN[1], M. E. LARKUM[12], A. GRUART[6], H. BADING[10], R. SPRENGEL[2], V. GRINEVICH[13], J. M. DELGADO-GARCIA[6];
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Abstract: A fear memory is formed rapidly and can last a lifetime. Different brain regions participate in organizing fear circuits. However, it is largely unknown how fear memory circuits are formed and printed across the different brain regions and which circuit pathways are activated during retrieval. With advanced genetic approaches, we interrogated lateral/basolateral amygdala (LA/BLA) and medial prefrontal cortex (mPFC) in fear memory formation and expression by blocking synaptic plasticity and synaptic output before and after fear conditioning, and tagged activated cells for optogenetic memory recall. We found that both presynaptic mPFC and postsynaptic LA/BLA NMDARs are required for the formation of cued fear memory and

printing of memory engrams in different brain regions. Our results provide compelling evidence that NMDAR dependent synaptic plasticity facilitates systems consolidation for the sequential printing of fear memory engrams from LA/BLA to mPFC and, subsequently, to the other brain regions for flexible memory retrieval.

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Poster

243. Memory Engrams

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Topic: H.01. Animal Cognition and Behavior

Support: MQ-Mental Health Quality of Life Grant MQ15FIP100012

Title: Connectivity of engram cells during fear memory consolidation

Authors: *L. DIXSAUT, J. GRAFF;
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Abstract: The formation and storage of memories in the brain has been under deep investigation for several decades. Nevertheless, the precise molecular and cellular mechanisms by which memories are initially encoded and subsequently stored - in the same or different brain areas - are still to be investigated. Recent evidence has suggested that both the hippocampus (HIP) and the medial prefrontal cortex contain engram cells already at encoding, but whereas the HIP engram is already active at learning, the mPFC one is kept silent until the memory is fully consolidated. Thereafter, however, natural recall cues no longer activate the HIP engram but only the mPFC one (Kitamura et al., 2017). In this project, we aim to identify putative upstream key regions playing a role in this HIP to mPFC engram shift. Using AAV retrograde tracing in mice combined with immediate early gene quantification throughout the different phases of contextual fear memory formation and consolidation, our data suggest that projections from the claustrum to the entorhinal cortex and mPFC are involved in this consolidation process. These specific connections can then be manipulated chemogenetically in order to assess their functional role during the different memory phases. Collectively, we aim to further refine the working model of memory formation by deciphering the mechanisms of long-term systems consolidation in cortical structures.
Kitamura, T., Ogawa, S.K., Roy, D.S., Okuyama, T., Morrissey, M.D., Smith, L.M., Redondo,

R.L., and Tonegawa, S. (2017). Engrams and circuits crucial for systems consolidation of a memory. Science *356*, 73-78.

Disclosures: L. Dixsaut: None. **J. Graff:** None.

Poster

243. Memory Engrams

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Support: NIH Grant NIDCDR01

Title: Odor discrimination training enhances intracortical synaptic connectivity to stabilize neural ensemble representations in piriform cortex

Authors: *M. CANTO-BUSTOS, C. BASSI, S. F. MEHAN, K. FRIASON, A.-M. M. OSWALD;
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Abstract: The anterior piriform cortex (APC) decode olfactory stimuli by the joint activity of populations of neurons, or ensembles. However, in contrast to other cortical areas, neural activity lacks topographic organization for odor identity. One advantage of this architecture is a prominent capacity to represent different combinations of odorants by the activity of neuronal ensembles broadly distributed over the rostral-caudal extent of the APC. It is known that the formation and long-term stabilization of these ensemble representations are achieved through associative synaptic plasticity between co-activated Pyramidal Cells (PCs). In this study, we investigate the synaptic mechanisms that contribute to the reactivation and stabilization of odor evoked ensembles following discrimination training. Briefly, we use targeted recombination in active populations (TRAP) to tag neural ensembles that are active when mice reach criterion performance in an odor mixture discrimination task. We then re-evaluated task at 5, 10 and, 15 days post criterion and tagging. At each time point, we also invested the reliability of reactivation of the tagged ensembles using cFos immunochemistry. We find reactivation of ensemble neurons in trained animals (~30-50%) at all time points, but only <20% reactivation of odor evoked ensembles in untrained animals. This suggests that training enhances the stability of the ensemble responses to learned odors. We then tested whether training enhances within ensemble synaptic connectivity. We compared the intracortical synaptic inputs to tagged (tdTom+) versus non-tagged PCs using two methods. First, we electrically stimulated the intracortical fiber tract (L1B); and second, we expressed channelrhodopsin (ChR2) in a subset of tagged ensemble neurons. In trained animals, tagged ensemble PCs (tdTom+) response to L1B stimulus showed excitatory postsynaptic potentials (EPSPs) ~85% higher, and excitatory

spontaneous activity (sESPCs) ~75% more frequent compared to unlabeled PCs outside of the ensemble. Likewise, within ensemble PCs received stronger, optically evoked excitatory synaptic inputs from other ChR2+ ensemble neurons compared to PCs outside of the ensemble. On the other hand, there were no differences in synaptic strength between ensemble PCs (tdTom+) and unlabeled PCs following novel odor exposure. Altogether, our findings suggest that piriform cortex ensembles recruited during trained odor-guided behavior are stabilized by long-term enhancement of intracortical synaptic connectivity between ensemble neurons.

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Poster

243. Memory Engrams

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Support: NIH Grant NS086960

Title: Two parallel ensembles within a distributed neocortical circuit encode essential information for an advanced cognitive task, visual shape discrimination learning

Authors: *A. I. GELLER, A. NAGAYACH, M. GHAFARI, Y. ZHAO, G. S. COLLINS, A. SINGH;
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Abstract: Neural network and synaptic plasticity theories hypothesize that essential information for advanced cognitive tasks is encoded in neuronal ensembles in distributed neocortical networks. Here, we show that two parallel ensembles, each spanning multiple neocortical areas, encode essential information for an advanced cognitive task, visual shape discrimination learning.
We studied a circuit that contains a critical multimodal associative area, postrhinal (POR) cortex, and encodes this learning. Several hundred glutamatergic and GABAergic neurons in POR cortex received a constitutively active protein kinase C (PKC) (J Neurosci 2005 25 8468-81). This intervention activates specific PKC pathways with critical roles in synaptic plasticity, increases activation-dependent neurotransmitter release, and enhances accuracy for new visual shape discriminations.
Some of the essential information is encoded in the genetically-modified, local circuit in POR cortex (PNAS 2010 107 14478–83). After gene transfer and learning new discriminations, the circuit in POR cortex was ablated by lesioning: The lesions selectively reduced performance for discriminations learned after gene transfer. Correlatively, during learning, activity is increased in

the genetically-modified circuit, as shown by activity-dependent gene imaging. This local circuit is sparse coded and small, ~500 neurons.
Both learning and recall require the activity of an identified ensemble within this local circuit, the transduced neurons (Hippocampus 2019 1-16). We blocked fast neurotransmitter release from these neurons by coexpressing a Synaptotagmin I (Syt I) siRNA and the PKC, resulting in deficits in learning and recall. Further, specific signaling pathways required for learning were activated in this ensemble; required pathways include CaMKII, MAP Kinase, CREB, and dendritic protein synthesis.
Now we show that two parallel circuits, which include the required ensemble in POR cortex, each encode essential information. First, we delivered the PKC into neurons in POR cortex. Second, the rats learned new discriminations. Third, we blocked activity in connected postsynaptic neurons in either perirhinal (PER) cortex or TEv. For this intervention, we used gene transfer to connected neurons (Brain Res 2012 1473 173-84) to deliver the Syt I siRNA into connected neurons in either PER cortex or TEv. This intervention blocked the CREB and dendritic protein synthesis pathways, blocked neuronal activity, and caused deficits in performance. Thus, two parallel ensembles within a neocortical circuit each encode essential information for performing an advanced cognitive task.

Disclosures: **A.I. Geller:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alkermes. **A. Nagayach:** None. **M. Ghafari:** None. **Y. Zhao:** None. **G.S. Collins:** None. **A. Singh:** None.

Poster

243. Memory Engrams

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 243.21/X29

Topic: H.01. Animal Cognition and Behavior

Support: ANR Hippencode
FRM Equipe

Title: Intracellular dynamics of CA3 pyramidal cells supporting sparse coding during theta and large irregular activity

Authors: *C. MULLE, A. KEES, M. MALÉZIEUX;
Interdisciplinary Inst. For Neurosci., Bordeaux, France

Abstract: Wakefulness is comprised of distinct brain states, each correlated with different behaviors and characterized by specific oscillatory patterns in the local field potential (LFP). Pyramidal cells in area CA3 of the hippocampus, which are involved in rapid encoding of single-trial memory, have different firing properties during different brain states. During exploratory

behaviors, which are associated with memory encoding, the hippocampal LFP displays theta oscillations and CA3 pyramidal cells fire according to the location of the animal. In contrast, during quiet wakefulness, the hippocampal LFP displays large irregular activity punctuated by short oscillations known as sharp-wave ripples, which play a role in memory consolidation. Here, specific ensembles of cells 'replay' previously formed sequences. In both states, however, the activity of cells is sparse, which is likely important for memory processing. We hypothesize that changes in intracellular properties of CA3 PCs may ensure sparse coding during different brain states and underlie the ability to support the associated memory processes. To explore this, we made whole-cell patch-clamp recordings from CA3 pyramidal cells in awake head-fixed mice, and characterized brain states using measurements of running speed and CA3 LFP. Overall, we find evidence for mechanisms of sparse coding occurring in both theta and LIA, but the nature of these mechanisms differ between the two states. Specifically, we find that during theta, most cells consistently hyperpolarize and reduce their firing rate, while very few consistently depolarize and increase their firing rate. This coincides with a decrease in variance in the membrane potential and no change in the firing threshold relative to the membrane potential. These results are consistent with the hypothesis that a global inhibitory process shuts down most CA3 pyramidal cells and ensures that increased excitatory inputs result in increased firing in only a few cells. In contrast, despite the fact that LIA events tend to coincide with a depolarization, few cells do so consistently and thus most cells have a variable response to LIA. All cells have increased variance in the membrane potential, implying an increased effect of synaptic input. Additionally, the overall membrane potential is further from spike threshold during LIA. These results are consistent with the hypothesis that an increase in the relative firing threshold counteracts a global increase in input and thus only the few cells that experience a depolarization increase firing during LIA. Thus, the coding remains sparse despite the increased level of activity on the synaptic and network levels.

Disclosures: C. Mulle: None. **A. Kees:** None. **M. Malézieux:** None.

Poster

244. Hippocampus and Cognition

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 244.01/X30

Topic: H.01. Animal Cognition and Behavior

Support: Council of Scientific and Industrial Research (CSIR) India
DBTO/BCN/BJ/0402
DSTO/BCN/BJ/1102
DSTO/BCN/BJ/1297
JTT/MUM/INST/IIOS/2011314/0033

Title: Generalisation of temporal memory in rodents

Authors: *S. SHRIDHAR[1], V. P. SINGH[2], S. KUNDU[3], R. SHARMA[3], B. JAYAPRAKASH[4]; [1]Ctr. for Neurosci., Indian Inst. of Science, Bengaluru, Bengaluru, India; [2]Ctr. for Neurosci., [3]Indian Inst. for Sci., Bengaluru, India; [4]CNS, Indian Inst. of Sci. Malleshwaram Bangalore, Bangalore, India

Abstract: Episodic memory is remembering the what, when and where of an autobiographical event. We know that over time, memories might lose the richness of detail, and generalize contextual information and presented stimuli. However, very little is known about the nature of temporal aspects of these memories and to what extent they are preserved and generalized over time. Our current research aims to understand the evolution of temporal memory, specifically that of temporal order memory. To this end we have designed novel behavioural paradigms, through which we can independently retrieve and test the different aspects of temporal memory in C57/BL6 mice. These paradigms include an Order-Place association task (in an event arena) and a compound tone fear conditioning task involving Tone-Order discrimination. These paradigms are designed such that they can be contrasted with comparable, established paradigms that lack an explicit temporal component. Further, these comparisons are important, since in these non-temporal tasks, the precise time scales of consolidation are known. By probing for temporal-order memory at various time-points during the training/learning phase and after, where we attempt to quantify how much of the memory persists, we have been able to observe how this temporal-order memory evolves with time.

Disclosures: S. Shridhar: None. **V.P. Singh:** None. **S. Kundu:** None. **R. Sharma:** None. **B. Jayaprakash:** None.

Poster

244. Hippocampus and Cognition

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 244.02/X31

Topic: H.01. Animal Cognition and Behavior

Title: Differential effects of energy dense diets on object recognition memory in young adult male and female Long Evans rats

Authors: *S. L. NEESE, V. SUKOLOWSKY, P. ORT, J. RHOADES;
Cornell Col., Mount Vernon, IA

Abstract: Obesity rates continue to rise in Western populations, due in part to the availability of energy-dense foods that are high in fats and sugars. In particular, obesity now affects 17% of children and adolescents in the U.S with this upward trend expected to continue. Little research

has established the sexually-dimorphic behavioral changes that might accompany intake of high fat or carbohydrate foods during development. In addition, inconsistent changes in weight and fasting glucose levels have been reported in adulthood following high carbohydrate exposure in the Long-Evans (LE) strain of rats. The current study explored the isolated impact of maintenance on either a diet high in fat (60% of calories from fat sources) or a diet high in carbohydrates (32% sucrose w/v) in developing male and female LE rats. Control rats received a low fat diet (10% of calories from fat sources) or plain water, respectively. Twenty-one day old rats were weaned and pair-housed by sex and provided on either a high fat or high carbohydrate diet for 8 weeks. Rats were then tested in a hippocampally-sensitive novel object recognition task. Briefly, rats were given 5 minutes to explore 2 identical objects located within a testing apparatus. After a one-hour delay one object was replaced with a new object and interaction with each object was recorded for an additional 5 minute time period. Male rats exposed to a high fat diet showed impaired memory for objects following a one-hour delay compared to low fat diet exposed rats, an effect not seen in the female rats, even though both male and female rats fed a high fat diet had increased body weights compared to low fat exposed rats ($p<0.05$). Conversely, maintenance on a high carbohydrate diet failed to impact object memory in male or female rats ($p>0.05$). In addition, fasting glucose measures and body weights at the time of testing in the high carbohydrate treated rats were not significantly different from those in the control group, suggesting a differential effect of high carbohydrate exposure on metabolic measures in LE rats during development. Together, these findings suggest that developmental exposure of LE rats to a high fat diet alters body weight and impairs object memory in a sex-specific manner.

Disclosures: **S.L. Neese:** None. **V. Sukolowsky:** None. **P. Ort:** None. **J. Rhoades:** None.

Poster

244. Hippocampus and Cognition

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 244.03/X32

Topic: H.01. Animal Cognition and Behavior

Support: Exploratory Research for Advanced Technology (JPMJER1801)
Precursory Research for Embryonic Science and Technology (JPMJPR1785)
KAKEN 19J01068

Title: Encoding of elapsed time in the order of minutes by the hippocampus and striatum

Authors: *Y. SHIKANO[1,2], Y. IKEGAYA[2], T. SASAKI[2];
[1]Sch. of Med., Keio Univ., Tokyo, Japan; [2]Pharmaceut. Sci., Univ. of Tokyo, Tokyo, Japan

Abstract: Animals need to recognize the temporal context of the events based on previously encoded memories with a variable time range. Previous reports have shown that neuronal spike

activity in the hippocampus, neocortex, and striatum, represent the elapsed time on the timescale of tens of seconds. However, neuronal spike activity responsible for processing longer timescales such as minutes remains elusive. Here, we established a novel behavioral task in which rats stayed in a small task box with a feeding port at a specific corner. Single 45-mg food pellets were given to the rats if they peeked in the feeding port during 2-3 s pellet presenting periods every five minutes. This experimental paradigm enabled us to study the relationship between animal's explicit timing behavior for predictable feeding events and temporal patterns of neuronal spiking activity over minutes-range timescale. Utilizing the small task box could reduce the contribution of variable locomotion and locations on neural signals. Peek behavior was continuously monitored and quantified as a behavioral correlate of the temporal estimate. Bilateral injection of muscimol into the hippocampus decreased temporal information. Next, spike activity was recorded from the dorsal hippocampal CA1 area and dorsal striatum during the task. We found that a subset of neurons showed temporal changes in firing patterns in each 5-min trial. We therefore introduced LN model (Hardcastle et al., 2017) for detailed analyses of unit activity. As a result, characteristic spike patterns were not simply explained by animals' peeking behavior, positions, or running speed. By Bayesian decoding, population spike data predicted the elapsed time more significantly than shuffle spike data. Our results provide evidence that hippocampal and striatal neurons periodically and repeatedly represent the elapsed time to cover a duration on a time scale of minutes.

Disclosures: **Y. Shikano:** None. **Y. Ikegaya:** None. **T. Sasaki:** None.

Poster

244. Hippocampus and Cognition

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 244.04/X33

Topic: H.01. Animal Cognition and Behavior

Support: The Swedish Research Council (M-2009-52)
Gunvor och Josef Aners Foundation
The Swedish Society of Medicine (FOA10H-031)
The Swedish Brain Foundation
The National Board of Health and Welfare (SLS-173121)

Title: Dopamine in the ventral hippocampus signals novelty

Authors: J. TITULAER[1], C. BJORKHOLM[2], K. FELTMANN[2], B. SCHILSTROM[2], ***A. K. KONRADSSON-GEUKEN**[1];
[1]Uppsala Univ., Uppsala, Sweden; [2]Karolinska Institutet, Stockholm, Sweden

Abstract: Introduction: Both the prefrontal cortex (PFC) and the hippocampus are innervated by dopamine (DA) cells originating from the ventral tegmental area (VTA), and a plethora of evidence shows the crucial importance of dopaminergic signaling in the PFC and the hippocampus for efficient cognitive function. Previous studies investigating DA in response to novelty have mainly investigated DA release in response to novel surroundings. However, a novel environment could enhance catecholamine release for several reasons other than novelty detection per se. Taken together, the role of DA in the PFC and hippocampus concerning novelty remains to be determined.
Methods: Microdialysis was combined with the novel object recognition test in order to investigate the role of DA and norepinephrine (NE) release in the medial PFC (mPFC) and hippocampus on recognition memory. After a stable baseline, a novel object was introduced in the experimental cage. The object remained in the cage with the animal for the duration of 1 microdialysis sample (30 minutes), after which it was removed. 2 hours later, either a familiar or a novel object was presented to the rat at the same location in the experimental cage for 30 minutes.
Results: Introduction of a novel object (i.e. the first object) significantly increased hippocampal DA- and NE release compared to baseline values. Two hours after the introduction of the first object, a second object was placed in the same position as the first object. In the hippocampus, introduction of a novel object again induced a significant increase of DA and NE release, compared to the effect of a familiar object, whereas this did not occur in the mPFC.
Discussion: In the present study, we show that DA is released in the ventral hippocampus, but not the mPFC specifically in the response to novel objects. This corroborates the role of DA proposed by Listman and Grace, as DA is signaling novelty in the hippocampus and subsequently acting to facilitate the formation of long-term memories [1]. Moreover, the observation that DA is released in the ventral hippocampus, but not in the mPFC, in response to novelty suggests a crucial role for hippocampal DA not only in the acquisition of memories but also for the consolidation of recognition memory. Furthermore, we demonstrated the feasibility of measuring neurotransmitter release in response to novelty without the confounders of using a novel environment. Thus, this paradigm could be used to further investigate the temporal aspects of the neurotransmitter release in detection and response to novelty.**References**: 1 Lisman JE, Grace AA., 2005. Neuron. 46(5):703-713.

Disclosures: J. Titulaer: None. **C. Bjorkholm:** None. **K. Feltmann:** None. **B. Schilstrom:** None. **A.K. Konradsson-Geuken:** None.

Poster

244. Hippocampus and Cognition

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 244.05/X34

Topic: H.01. Animal Cognition and Behavior

Support: NSF Neurophotonics Research Traineeship (NRT) DGE-1633516

Title: Effects of theta phase specific modulation of engram neurons in mice hippocampus on memory recall

Authors: *B. RAHSEPAR[1], K. GHAEMI[1], J. NOUEIHED[1], B. LAHNER[1], M. H. QUICK[1], S. RAMIREZ[2], J. A. WHITE[1];
[1]Biomed. Engin., [2]Boston Univ., Boston, MA

Abstract: Memory processing in the mammalian brain is a dynamic cognitive task requiring coordination between various brain regions, including hippocampus. The theta rhythm, a 4-12 Hz oscillation, is observed during memory processing. Behavioral studies suggest that theta oscillations contribute to episodic memory. Furthermore, a prominent computational model, Separate Phased of Encoding and Recall (SPEAR), based on in vitro electrophysiology and anatomical data, proposes that different phases of theta (peak versus trough) are used to separate recall of stored memories from encoding of new experiences, in a form of temporal multiplexing. The discovery of hippocampal engram cells, a sparse population of molecularly identifiable neurons that are highly active during memory formation and appear both necessary and sufficient for memory recall, gives us the opportunity to explore memory formation from a causal, mechanistic perspective. Using real-time optogenetic stimulation of engram neurons, we aim to identify temporal dynamics of engram neurons, the role of the theta rhythm, and these factors' contributions to formation and recall of memories. Mice were injected bilaterally with cFos-tTA and tTA-ChR2-EYFP in dentate gyrus (DG) and implanted with bilateral fiber optics in DG and an LFP electrode in the CA1 region of the hippocampus. They were exposed to *context A* and received four electrical foot shocks. Post-fear-conditioned animals were re-exposed to the fearful *context A* as well as a novel *context B.* They exhibited high level freezing in *context A,* corresponding to recall of the fearful memory, while exploring naturally in *context B.* Later, engram neurons were optogenetically stimulated in *context B* with three different stimulation parameters: constant 20-Hz stimulation, theta peak stimulation, and theta trough stimulation. Our initial results indicate that optogenetic stimulation during the hypothesized recall phase does in fact maximize recall, and that theta-paced stimulation induces recall more effectively than does traditional 20-Hz stimulation.

Disclosures: B. Rahsepar: None. **K. Ghaemi:** None. **J. Noueihed:** None. **B. Lahner:** None. **M.H. Quick:** None. **S. Ramirez:** None. **J.A. White:** None.

Poster

244. Hippocampus and Cognition

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 244.06/X35

Topic: H.01. Animal Cognition and Behavior

Support: ERC Starting Grant
Israel Science Foundation, Individual research grant
Canada-Israel grants (CIHR-ISF)
Azrieli Fellowship

Title: Structure, neuronal content, and functional significance of hippocampal astrocytic domains

Authors: *A. DORON, R. REFAELI, A. BENMELECH-CHOVAV, T. KREISEL, I. GOSHEN;
Hebrew Univ., Jerusalem, Israel

Abstract: Recent ground-breaking research has revealed that astrocytes have a significant role in modulating neuronal activity and even behavior. Previous studies have shown that astrocytes cover discrete physical domains, with minimal overlap between their fine processes, but these works were limited in the number of cells that were investigated. Moreover, the possible functional significance of this tightly-controlled spatial organization has been speculated upon, but never directly explored. Here, we provide a comprehensive structural characterization of astrocytic domains and their neuronal contents in the hippocampus. We simultaneously expressed one fluorophore in distinct neuronal populations (CaMKII/Parvalbumin/Somatostatin/VIP), and another in astrocytes, then cleared the brains using the CLARITY technique, and imaged whole hippocampi. We reconstructed the elaborate morphology of the astrocytes and detected the neuronal somas residing in their domains, providing the first comprehensive quantification of the distribution of four neuronal sub-types in 3D hippocampal astrocytic domains. Next, to investigate a possible functional role of astrocytic domains in the selection of memory neuronal ensembles, we expressed one fluorophore in astrocytes and concomitantly tagged activated neurons during different behaviors (home-cage, fear conditioning, and novel environment exposure). This allowed us to detect the distribution of active neurons in astrocytic domains, and show their role in memory allocation. Finally, to study the real time activity of neurons belonging to the same astrocytic domain, we used 2-photon imaging of CA1 pyramidal neurons in mice exploring a virtual reality maze, and tested their activity as a function of location in the virtual environment. Additionally, we conducted a z-scan to image astrocytes whose processes cross the imaging plane, and then reconstructed their 3D structure. We then attributed each imaged neuron to a single astrocytic territory, and tested the dynamics of space representation among neurons belonging to the same domain. In summary, we present a broad anatomical characterization of astrocytic domains in the hippocampus, and provide evidence indicating their relevance to neuronal activity during cognitive tasks.

Disclosures: A. Doron: None. **R. Refaeli:** None. **A. Benmelech-Chovav:** None. **T. Kreisel:** None. **I. Goshen:** None.

Poster

244. Hippocampus and Cognition

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 244.07/X36

Topic: H.01. Animal Cognition and Behavior

Title: Does spatial memory require hippocampus during orienting behavior?

Authors: *N. P. SIDELL[1], E. L. NEWMAN[3], K. BLANKENBERGER[2];
[1]Dept. of Psych. and Brain Sci., Indiana Univ., Bloomington, IN; [2]Indiana Univ., Dept. of Psych. and Brain Sci., IN; [3]Dept. of Psych. and Brain Sci., Indiana Univ. Bloomington, Bloomington, IN

Abstract: Spatial memory is essential for an organism's ability to survive. Spatial memory tasks, such as the 8-arm delayed-win-shift task used with rats, require an intact hippocampus. Here, we sought to better understand when during the task the hippocampus is required specifically. Our working hypothesis was that intact hippocampal activity is particularly necessary during orienting behaviors such as rearing. This hypothesis was motivated by published evidence showing that orienting drives place field formation and unpublished data from our lab showing a correspondence between rearing behavior and task performance. To test this hypothesis, we tested if rats' performance on the 8-arm delayed-win-shift task decreased when the dorsal portion of hippocampal subregion CA1 (dCA1) was inactivated during rearing behavior. To do this, we implemented a closed-loop system to temporarily silence dCA1 via optogenetic inhibition during rearing events. Rats performed one trial a day wherein four arms were randomly shown during a study phase and must visit the remaining four arms 4 minutes later in the test phase. Across different days, we either inactivated dCA1 during any and all rearing in the study phase (inactivation condition) or performed no inactivation (control condition). In neither condition was inactivation performed during the test phase. Preliminary results (n=4) show that, relative to the control condition, performance at test was significantly reduced during the inactivation condition. This indicates that the inactivation impaired encoding of the spatial memories needed to support performance at test. Analysis of the total time spent rearing during the study phase revealed a significant increase in the amount of rearing in the inactivation condition compared to
1
the control condition. We suggest this increase indicates that inactivating dCA1 during rearing impaired the rats' ability to reap the functional benefit of rearing, thereby motivating additional rearing. Data collection remains ongoing and new conditions are being added to determine if the effects are unique to performing the inactivation during rearing behavior. Results from these efforts will be presented on the poster.
2

Disclosures: **N.P. Sidell:** None. **E.L. Newman:** None. **K. Blankenberger:** None.

Poster

244. Hippocampus and Cognition

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 244.08/X37

Topic: H.01. Animal Cognition and Behavior

Support: JPB
HHMI
RIKEN

Title: Hippocampal circuit mechanisms encoding experience as abstract, ordered, and transferable chunks

Authors: ***C. SUN**[1], W. YANG[3], J. MARTIN[1], S. TONEGAWA[2];
[2]The Picower Inst. for Learning and Memory, [1]MIT, Cambridge, MA; [3]Neurosci., NYU, New York, NY

Abstract: During continuous daily experience, individuals automatically subdivide the experience into discrete chunks (events). But the neural basis for representing episodic experience as a sequence of discrete chunks separate from the continuous experience remains understudied. Here, we investigate a hippocampal CA1 “chunking code” tracking an episode as discrete chunks (events) and the sequential relationships between them. The chunking code is unaffected by unpredicted variations within the events, and therefore treats event identity as a fundamental organizing unit. The chunking code and continuous spatial code (and even continuous time code) are represented in the same cells in this dual code manner, but can be independently perturbed. Optogenetic inactivation of MEC inputs to CA1 disrupts the chunking but not spatial code. The inactivation of CA2 and CA3 inputs to CA1 were also examined, to investigate the contribution of the main upstream brain regions to the chunking code. The chunking code tracking abstract chunks of experience may be one of the fundamental codes for representing an episode, alongside codes tracking continuous changes.

Disclosures: **C. Sun:** None. **W. Yang:** None. **J. Martin:** None. **S. Tonegawa:** None.

Poster

244. Hippocampus and Cognition

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 244.09/X38

Topic: H.01. Animal Cognition and Behavior

Support: NIH R01MH110311

Title: Mnemonic and spatial representations in primate retrosplenial cortex

Authors: *N. A. KAMBI[1], J. M. PHILLIPS[2], M. J. REDINBAUGH[2], S. MOHANTA[3], B. WANG[4], S. CHANNABASAPPA KENCHAPPA[5], Y. B. SAALMANN[6];
[2]Psychology, [1]Univ. of Wisconsin-Madison, Madison, WI; [3]Univ. of Wisconsin, Madison, Madison, WI; [4]Univ. of Wisconsin, Madison, WI; [5]American Family Insurance, Madison, WI; [6]Univ. of Wisconsin - Madison, Madison, WI

Abstract: Episodic memory involves the storing of events, locations and context of personal experiences in their varied details. Retrosplenial cortex (RSC), along with the hippocampus and anterior thalamus, are part of a neural network thought to support episodic memory. The RSC also has robust bidirectional connections with frontal and parietal association areas. Although this anatomical connectivity positions RSC as an important network hub, its precise functional contributions are unclear. Human neuroimaging studies indicate that RSC codes for permanent landmarks, or internal spatial representations with respect to landmarks, in navigation tasks and during scene processing. To support such spatial representations and their transformations, rodent studies suggest that the RSC acts as an intermediary between different spatial frames of reference in the parietal cortex (egocentric) and hippocampus (allocentric). These processes may be related to RSC's role in episodic memory, considering that lesions to RSC in monkeys cause memory recall deficits in an object-in-place task. However, it is not clear how primate RSC neurons represent remembered scenes.

We used laminar probes to record spiking activity from RSC cells (n=101) in 2 macaques as they performed a context- or scene-guided memory task. Scenes consisted of two characters (one target and one distracter) and a larger, colored object that served as a landmark. The animal had to learn by trial and error to saccade to the target to earn a juice reward. Four new scenes and an old well learnt fifth scene was presented daily. The target location depended on integration of the specific scene contents. After learning we presented recall (requiring saccade to remembered location of target), rotated (requiring spatial transformation) and cued recall (saccade to placeholders at the target location) versions of the scenes. We used linear regression analysis to quantify how different scene contents influenced RSC spiking activity.

RSC cells showed sensitivity to multiple aspects inherent to the task. 64% of cells showed scene selectivity. Many RSC cells responded to specific scene contents as well: landmarks influenced

49%, target identity influenced 62%, and target location influenced 54% of cells. 35% of cells distinguished the rotated versus unrotated versions of scenes. Finally, 53% of cells selectively responded to newly learnt versus old scenes.
These preliminary results suggest that RSC not only contributes to the memory of past experiences, but also integrates information across different temporal and spatial contexts, as well as frames of reference, for successful retrieval of those experiences.

Disclosures: **N.A. Kambi:** None. **J.M. Phillips:** None. **M.J. Redinbaugh:** None. **S. Mohanta:** None. **B. Wang:** None. **S. Channabasappa Kenchappa:** None. **Y.B. Saalmann:** None.

Poster

244. Hippocampus and Cognition

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 244.10/X39

Topic: H.01. Animal Cognition and Behavior

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DBTO/BCN/BJ/0402
DSTO/BCN/BJ/1102
DSTO/BCN/BJ/1297
JTT/MUM/INST/IIOS/2011314/003

Title: Vector maps of flow is sensitive in determining the centre of intended search, uncertainty surrounding the search and the absolute error associated with the spatial memory in water maze

Authors: *M. PRABOD KUMAR[1], D. MEHROTRA[1], N. NRUTHYATHI[1], B. JAYAPRAKASH[2];
[1]Indian Inst. of Sci., Bangalore, India; [2]neurobiology, Indian Inst. of Sci. Malleshwaram Bangalore, Bangalore, India

Abstract: Conventional behavioral measures for testing hippocampal dependent memory using Morris water maze involves comparing the residence time of the mice across different quadrants. Such measures are inherently simple and thereby are limited in their ability to extend these behavioral task to ask finer question about the nature of the memory. Several modifications to the analysis have been proposed and various measures have proposed to enhance the sensitivity of this task. Here we use the vector fields to describe the search pattern of the mice and develop quantitative measures that are intuitive as well as sensitive to measure the degree of impairment in the memory than just identifying if there is an impairment. Further, using such an approach allows one to investigate the nature of the strategy used by the animals to navigate to the target location. Use of vector fields allowed us to describe the observed search pattern in terms of curl, divergence and other vector field properties. We propose that in such a description the

convergence of the flow vectors would serve as the measure of certainty about the target location while the curl of such a vector field represent the putative epicentres of search.

Disclosures: **M. Prabod Kumar:** None. **D. Mehrotra:** None. **N. Nruthyathi:** None. **B. Jayaprakash:** None.

Poster

244. Hippocampus and Cognition

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 244.11/X40

Topic: H.01. Animal Cognition and Behavior

Support: R01MH102394
P20 GM103446
IIA-1301765

Title: Optogenetic suppression of the medial septum impaired choice accuracy on a working memory dependent conditional discrimination task

Authors: ***Z. M. GEMZIK**, A. L. GRIFFIN;
Univ. of Delaware, Newark, DE

Abstract: Spatial working memory (SWM) is the ability to process and maintain spatially-relevant, goal-directed information over a temporal gap, and has been shown to rely on an intact hippocampus (HPC). One of the most prominent oscillations in the HPC is theta (5-12 Hz), which supports neuronal activity relevant to SWM guided behavior. The medial septum (MS) is necessary for the generation of theta oscillations in the HPC. To examine the role of the MS in SWM, we used an inhibitory optogenetic viral vector (AAV5-CAG-ArchT-tdTomato) to transduce MS neurons and suppress their activity during a SWM dependent visual-spatial conditional discrimination (CDWM) task on a T-maze. For this task, floor inserts that vary in texture and color serve as conditional cues for the rewarded goal arm. Because the floor insert cues extend only halfway up the central stem of the maze and are not available when the rat makes a goal-arm choice, the rats are required to remember the cue for a brief period of time in order to make a correct choice and receive food reward. Our results show a significant drop in choice accuracy with MS optogenetic suppression (light-on vs. light off, $t(5) = 6.05$, $p = 0.0009$). In contrast, there was no choice accuracy deficit when MS neurons were optogenetically suppressed as rats performed a SWM-independent variant of the conditional discrimination (CD) task. Choice accuracy was also not impaired when MS neurons were illuminated in the virus control group (AAV5-CAG-tdTomato). To further explore the choice accuracy deficit on the CDWM task, we compared the effects of MS suppression restricted to times when the rat traversed the portion of the stem containing the contextual cue to times when the rat traversed the

un-cued portion of the stem. In both cases, we found a significant drop in choice accuracy (cued light-on vs. light-off, $t(5) = 4.89$, $p = .002$) (un-cued light-on vs. light-off, $t(4) = 2.98$, $p = .03$). These findings demonstrate that the MS is necessary for both the encoding and maintenance of the cue information and for making a memory-guided decision. These data suggest that the MS equips HPC neurons with a temporal framework upon which to process and organize task-relevant information on a theta frequency timescale.

Disclosures: Z.M. Gemzik: None. **A.L. Griffin:** None.

Poster

244. Hippocampus and Cognition

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 244.12/X41

Topic: F.04. Stress and the Brain

Title: Raccoon brains by the numbers: Insights on problem-solving competency

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Abstract: The neocortex of the raccoon (*Procyon lotor*) is uniquely organized in accordance with the raccoon's diverse ecological niche profile and lifestyle characteristics (Krubitzer, 2007). In addition to species-specific cortical field distributions, raccoons are known for their flexible behavior and advanced problem-solving abilities (Pettit, 2010). In the current study, captive raccoons were exposed to a multi-access puzzle box to assess problem-solving ability. Animals that solved three solutions of the puzzle (n=7) were compared to raccoons that failed to solve any of the puzzle solutions (n=6). One hemisphere of each brain was prepared for isotropic fractionation, a technique used to quantify overall cell density (Herculano-Houzel & Lent, 2005). Following histological processing, cell counts in the somatosensory cortex, hippocampus, and prefrontal cortex were quantified, due to their potential contributing roles in problem-solving competency. Although no differences were observed in the somatosensory cortex, the high problem-solving group exhibited significantly more cells in the hippocampus than the low problem-solving group (x=35,237,500 and 51,472,917 respectively and p=0.007); processing of prefrontal cortical areas and neuronal vs. glial ratios to follow. In the next phase of processing, immunohistochemistry will be utilized to assess the number of brain-derived neurotropic factor (BDNF)-immunoreactive cells in the hippocampus; further, a small number of wild-caught brains (n=3) will be compared to sex-matched captive brains (n=3) to determine the effects of

captivity on cellular counts. Thus far, preliminary results suggest that area-specific cell counts provide relevant information about specific functional capacities (i.e., problem-solving ability). These findings are informative considering past findings of function-driven hippocampal modifications (e.g., larger hippocampus in food-storing birds; Krebs et al., 1989). Additional neurobiological research utilizing various animals characterized by unique neurobiological niches is necessary to provide information about functional outcomes associated with varying measures of brain structure and plasticity.

Disclosures: **S. Benson-Amram:** None. **M. Kent:** None. **S. Daniels:** None. **J.H. Drake:** None. **R. Fanelli:** None. **A.T. Gilbert:** None. **S.R. Johnson:** None. **A. Lai:** None. **A. Miller:** None. **N. Natale:** None. **S. Herculano-Houzel:** None. **K. Lambert:** None.

Poster

244. Hippocampus and Cognition

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 244.13/X42

Topic: F.04. Stress and the Brain

Support: NIH Grant MH116339
NIH Grant MH077681
Vanderbilt University Medical Center

Title: Exploring the role of ventral hilar mossy cells in social and aggressive behaviors

Authors: C. WILSON[1], S. LOWREY[3], M. E. JOFFE[2], P. CONN[2], M. PICCIOTTO[4], ***A. S. LEWIS**[3];
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Abstract: Mossy cells (MCs) are glutamatergic neurons with cell bodies located in the hilus of the dentate gyrus (DG). These cells project locally and also send projections distantly to contralateral and ipsilateral DG, with synaptic connections onto granule cell dendrites as well as interneurons. Recent studies have demonstrated the involvement of MCs in contextual encoding and seizure progression, though the role of MCs in many other behaviors involving the hippocampus is unknown. Our group and others have identified the dorsal DG (dDG) and hippocampus as important in the regulation of aggressive behaviors. Relatedly, we have conducted dDG calcium imaging demonstrating an inverse correlation between dDG activity and social approach behaviors in male mice. We hypothesized that ventral MCs (vMCs), which send strong projections to dDG granule cells, may be important in social and aggressive behaviors. To test this hypothesis, we used an intersectional targeting approach in male CD-1 mice to selectively express proteins of interest in ventral hilar MCs. We infused retrograde-pgk-Cre

adeno-associated virus (AAV) into unilateral dDG in conjunction with bilateral ventral hilar infusions of Cre-dependent AAVs expressing the activating DREADD hM3D(Gq) or mCherry to explore the effects of activating vMCs, or the calcium indicator GCaMP6f to quantify the activity of vMCs during behavior. This strategy selectively targeted vMCs, as demonstrated by colocalization between infected cells and GluR2/3, a marker of MCs, or calretinin, a marker of ventral but not dorsal MCs. Whole cell recordings were performed in hM3D(Gq)-expressing vMCs to confirm the activating effects of clozapine-N-oxide (CNO). Mice were then tested in the open field test, three-chamber sociability arena, and in the resident-intruder test with male C57BL/6 intruder mice, comparing behavioral performance after CNO (10 mg/kg) between mice expressing hM3D(Gq) or mCherry in bilateral vMCs. Similarly, vMC activity was recorded with fiber photometry during open field and resident-intruder interactions, demonstrating dynamic signal activity. These studies contribute to an increasing understanding of the hippocampus and its dorsoventral axis in regulation of social and aggressive behaviors.

Disclosures: C. Wilson: None. **S. Lowrey:** None. **M.E. Joffe:** None. **P. Conn:** None. **M. Picciotto:** None. **A.S. Lewis:** None.

Poster

245. Time Perception

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 245.01/X43

Topic: H.01. Animal Cognition and Behavior

Support: NIH NS100050

Title: Temporal scaling of neural trajectories in secondary motor cortex during a differential anticipatory timing task

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Abstract: The neural mechanisms underlying timing on the scale of seconds are not well understood. Of particular importance is the question of how neural circuits can produce the same motor output at different intervals. One can imagine two general mechanisms: temporal scaling of a pattern of neural activity (“scaled”) or a code in which, like a timer, different intervals are encoded by patterns of activity running at the same speed for shorter or longer durations (“absolute”). Here we address this issue by studying activity in secondary motor cortex (M2)—an area consistently implicated in timing—during a differential anticipatory task, in which rewards were delivered either 3 (Cue 1) or 6 (Cue 2) seconds after odor onset. After training, animals produced differential anticipatory distribution of licks: the mean lick onset times were

2.50 ± 0.04 s (Cue 1) and 4.77 ± 0.06 s (Cue 2) ($p < 0.001$). Furthermore, bilateral injection of a low concentration of muscimol (2 mM) in M2 produced a significant change in the lick onset time to Cue 1 and Cue 2 ($p < 0.001$). We next performed large-scale silicon probe recordings in M2 on well-trained animals. M2 neurons showed both spatially and temporally complex population activity during the task. To test if there is a code for time in M2, we ran a SVM decoder on population activity to decode the time between the cue onset and reward onset. Population activity in M2 could correctly decode elapsed time for Cue 1 (Pearson $R = 0.92 \pm 0.01$) and Cue 2 (Pearson $R = 0.81 \pm 0.03$) trials. We then examined the ability of the decoder trained on Cue 1 to decode absolute or scaled (i.e., compressed) time during Cue 2 (and vice-versa). The decoding performance in the scaled condition was significantly better than in the absolute condition ($p<0.001$ and $p<0.02$). Next we measured the pairwise Euclidean distance between the Cue 1 and Cue 2 neural trajectories across time, and extracted the corresponding time points at which the distance between both trajectories was minimal. The curves of the time index vs true time bin were again most consistent with scaling of the temporal code. To understand whether the scaled trajectories are accompanied by a change in neural gain or rather primarily reflect a change in speed of a trajectory we examined the normalized (i.e., firing rate of each neuron) Cue 1 and Cue 2 trajectories, which resulted in a stronger scaling of minimal distance. The notion that neural circuits can produce scaled trajectories to encode time is consistent with previous results (e.g. Mello et al, 2015; Wang et al, 2018), and we further established that the encoding of time in M2 is most consistent with "contracted" neural trajectory being traversed at slower speed for the longer interval.

Disclosures: S. Zhou: None. **D.V. Buonomano:** None. **S.C. Masmanidis:** None.

Poster

245. Time Perception

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 245.02/X44

Topic: H.01. Animal Cognition and Behavior

Title: Scalable representation of elapsed time with hippocampal cell assembly

Authors: *A. SHIMBO[1,2], E.-I. IZAWA[2], S. FUJISAWA[1];
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Abstract: From our daily life work to the survival of animal, spatial and temporal dimensions are essential information. In particular, using temporal information in seconds to minutes range, referred to as interval timing, is contributed to various behaviors, such as foraging, decision making, associative learning, and sequential motor learning. Recently, bodies of evidence that hippocampal neurons also represented temporal information were accumulated. Hippocampal pyramidal neurons activate successively between events (Pastalkova et al., 2009; MacDonald et

al., 2011), and these neurons are termed ‘time cell’ because of similarity of place cell. However, detailed properties of time cells during interval timing are largely unknown. To clarify this problem, we recorded activities of hippocampal neurons during the temporal discrimination task with manipulating length of durations. In this task, rats were required to discriminate two different interval, running on a treadmill, for selecting a correct choice to get water-reward and experienced different sets of intervals in task conditions, i.e. they experienced 5 s or 10s interval in block 1, 10 s or 20 s interval in block 2, and then 5 s or 10 s interval again in block 3. During the interval in which rats were required to measure elapsed time from a start of running, unit activities of dorsal CA1 neurons were recorded. A subset of the neurons showed sequential activities during the interval and selectively responded to same elapsed time in block1 and 3.When duration was enlarged in the block 2, majority of these cells enlarged their firing field and changed their firing-peak later. These results indicated that these neurons responded not the duration per se, but the percentage of duration, indicating that these cells show the scalable representation of the durations. Moreover, these hippocampal neural activities reflected the animal’s choice in the task indicating that the rats can use hippocampal neural activities to solve the task. Therefore, these results suggest that the hippocampus is a fundamental brain region for representing spatial and temporal dimensions.

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Poster

245. Time Perception

Location: Hall A

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Program #/Poster #: 245.03/X45

Topic: H.01. Animal Cognition and Behavior

Support: Marsden Fund UOO1212
Alexander von Humboldt Foundation

Title: Coordination of hippocampal phase precession is compromised in a maternal immune activation model of schizophrenia risk, providing a novel mechanism for disorganized temporal processing in schizophrenia

Authors: *L. J. SPEERS[1], K. R. CHEYNE[1], E. CAVANI[2], T. HAYWARD[1], R. SCHMIDT[3], D. K. BILKEY[1];
[1]Psychology, Otago Univ., Dunedin, New Zealand; [2]Max Planck Inst. for Biol. Cybernetics, Tubingen, Germany; [3]Dept. of Psychology, Univ. of Sheffield, Sheffield, United Kingdom

Abstract: Schizophrenia is a chronic, debilitating disorder with diverse symptomatology, including cognitive and motivational impairments. Disorganized temporal processing may be a trait marker of the disorder, with relevance to several classical symptoms. Activity in the

hippocampus, a region that encodes sequential information across time and space, has been shown to be disrupted in schizophrenia. Here we examined hippocampal phase precession, in a rodent Maternal Immune Activation (MIA) model of schizophrenia risk. Phase precession describes how individual place cells systematically advance their firing phase against the background theta oscillation as an animal moves through the cell's place field. Importantly, phase precession provides a mechanism by which the sequential order of progression across overlapping place fields can be compressed into a single theta cycle (known as a 'theta sequence'), a timescale suitable for the induction of synaptic plasticity and the storage of sequential information. However, accurate storage requires phase precession of individual cells to be coordinated across cell assemblies so that the phase onset and slope of precession is relatively consistent across cells. Without such coordination, theta sequences may become disorganized. Electrodes were implanted into the pyramidal cell layer of dorsal CA1 in mature rats that were the offspring of dams that had received either a single MIA (Poly I:C) or control injection (saline) during mid-gestation (GD15). Both cell firing activity and local field potentials were recorded as rats ran around a rectangular track. Our results showed that the within-cell slope and intensity of phase precession were similar for both groups, suggesting that the MIA intervention did not alter the ability to precess at the level of individual cells. However, a striking difference was observed across the population of MIA cells regarding the onset and subsequent phase of precession, with much greater between-cell phase variability in these animals. Furthermore, 'theta sequences' were significantly disorganized in MIA rats when compared to controls, most likely as a result of this variability. Together, these results strongly suggest that the coordination of activity across precessing cells is compromised in MIA animals, likely contributing to more disorganized processing and storage of sequential information. This finding provides, for the first time, evidence of a biological-level mechanism to explain disorganized temporal processing in schizophrenia, which could contribute to thought disorder, misattributions of agency or control, and impaired episodic memory and future planning.

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Poster

245. Time Perception

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Program #/Poster #: 245.04/X46

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R15DA039405

Title: Temporal expectations of methamphetamine vapor delivery

Authors: J. MORGAN-AGRICOLI, *M. S. MATELL;
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Abstract: The self-administration paradigm (SA) is an animal model that closely mimics human drug use, as drug delivery is contingent upon the subject's behavior. Most SA studies utilize a fixed-ratio schedule of reinforcement, allowing an assessment of the amount of work an animal will do for the drug. However, such procedures do not allow evaluation of the temporal expectation of drug delivery that may contribute to drug use and abuse. In the present study, we trained rats (n=4) to SA methamphetamine (MA) vapor (12.5-50 mg/ml propylene glycol) administered through computer controlled e-cigarette technology into an 850 cubic inch chamber under one L/min negative air pressure. Critically, rats were trained to self-administer on a fixed interval (FI) 20sec schedule of reinforcement. Following a trial initiation poke on the right nosepoke, the houselight was illuminated, and the first left nosepoke response at least 20 seconds after light onset was reinforced by a 2 second "puff" of MA vapor. Left nosepoke responses prior to 20 seconds had no programmed consequence. On average, the animals would initiate 5.92 trials per one-hour session, and complete 2.26 left nosepoke responses per initiated trial. The animals' mean left nosepoke response functions showed the scallop pattern that is typical of responding on an FI schedule. These data suggest that responding for MA can be brought under temporal control. Based on these pilot data we plan to add non-reinforced probe trials, thereby combining the SA procedure with the peak-interval timing procedure in order to assess the temporal specificity and precision of when the animal expects the drug (i.e., the location and spread of the peak) and how motivated the animal is to work for the drug (i.e., the height of the peak and the frequency of trial initiation). We will assess how temporal control changes over the course of an administration session, and over repeated sessions, as a function of MA exposure.

Disclosures: J. Morgan-Agricoli: None. **M.S. Matell:** None.

Poster

245. Time Perception

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 245.05/Y1

Topic: H.01. Animal Cognition and Behavior

Title: The generation of time in the hippocampal memory system

Authors: *E. T. ROLLS, P. MILLS;
Computer Sci., Oxford Ctr. for Computat. Neurosci., Coventry, United Kingdom

Abstract: We propose that ramping time cells in the lateral entorhinal cortex can be produced by synaptic adaptation, and demonstrate this in an integrate-and-fire attractor network model. Each attractor network has two populations of cells that alternate in their firing. There are three such

attractor networks, each operating with different frequencies of their oscillatory high periods of activity which then gradually decrease their firing rate. We propose that competitive networks in the hippocampal system can convert these lateral entorhinal time ramping cells into hippocampal time cells, and demonstrate this in a competitive network. We propose that this conversion is necessary to provide orthogonal hippocampal time representations to encode the temporal sequence of events in hippocampal episodic memory, and support that with analytic arguments (Rolls, 2016, 2018). We demonstrate that this processing system involving the lateral entorhinal cortex and its coupling to the hippocampus can produce hippocampal neuronal ensembles that not only show replay of the sequence later on, but can also do this in reverse order to produce reverse replay. The replay is in real time, but could be learned by hippocampal networks to produce rapid replay and reverse replay. This research addresses a major issue in neuroscience, the mechanisms by which time is encoded in the brain, and how the time representations are then useful in the hippocampal memory of events and their order.

Rolls,E.T. (2016) Cerebral Cortex: Principles of Operation. Oxford University Press: Oxford.
Rolls, E. T. (2018) The storage and recall of memories in the hippocampo-cortical system. Cell and Tissue Research 373: 577-604.
Rolls, E. T. and Wirth, S. (2018) Spatial representations in the primate hippocampus, and their functions in memory and navigation. Progress in Neurobiology 171: 90-113.

Disclosures: E.T. Rolls: None. **P. Mills:** None.

Poster

245. Time Perception

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Program #/Poster #: 245.06/Y2

Topic: H.01. Animal Cognition and Behavior

Support: CONACYT Grant 236836
CONACYT Grant 196
PAPIIT Grant IN202317-2

Title: Encoding of time and space magnitudes in the frontal lobe of the Rhesus monkey

Authors: *G. MENDOZA[1], J. C. MENDEZ[2], H. MERCHANT[1];
[1]Inst. de Neurobiologia UNAM Juriquilla, Queretaro, Mexico; [2]McGovern Inst. For Brain Res., Cambridge, MA

Abstract: Time and space are the dimensions in which behavior evolves. As a consequence, it is vital for individuals to capture both classes of information. Based on behavioral, neuropsychological and fMRI evidence, the existence of a common magnitude system in the brain, devoted to the processing of time, space and numerosity, has been proposed. To test if this

is true for the perception of time and space, we trained Rhesus monkeys to categorize as either ‘short' or ‘long' the interval or the distance between visual stimuli by selecting a conditional motor response. In alternate blocks of trials, monkeys categorized one of several stimuli sets that covered a wide range of intervals or distances (three interval sets and three distance sets). The shortest four magnitudes in each set had to be categorized as ‘short’ and the remaining four as ‘long’. Crucially, within each dimension —time or space— the sets were partially overlapped such that one particular interval or distance (absolute magnitude) acquired different relative values (short or long) depending on the context (block of trials) in which it was presented. We recorded neural activity in the pre-supplementary motor (pre-SMA) and dorsolateral prefrontal (DLPFC) cortices, areas previously shown to participate in perceptual categorization and interval timing. Analyses from Information and Signal Detection theories showed that the activity of neurons in these areas sequentially encoded, in each trial, the relevant stimulus magnitude as well as the category selected by the monkeys. The relationship between stimulus magnitude and the neuron’s firing rate was best described as a linear positive or negative function. This encoding was relative, i.e., across different sets of stimuli, the maximum firing rate was ‘normalized’. In turn, the selected category was encoded by category-selective neural activity. In DLPFC, neurons preferably encoded space magnitude, and the reverse was true for pre-SMA. Importantly, more than 50 % of the magnitude-related cells encoded both time and space. These results support the notion that the DLPFC and the pre-SMA are processing nodes for general magnitudes, namely, spatial and temporal quantities, as well as for the categorical representation of such information.

Disclosures: G. Mendoza: None. **J.C. Mendez:** None. **H. Merchant:** None.

Poster

245. Time Perception

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 245.07/Y3

Topic: H.01. Animal Cognition and Behavior

Support: PAPIIT Grant IN202317

Title: Decoding the tapping tempo from simultaneously recorded cell populations in the primate medial premotor cortex during beat entrainment to auditory or visual metronomes

Authors: *A. BETANCOURT-VERA, J. A. GAMEZ, G. MENDOZA, H. MERCHANT; Inst. de Neurobiologia UNAM, Campus Juriquilla, Queretaro, Mexico

Abstract: The ability to entrain to the beat of music is a hallmark of musical cognition. We know that tapping to a regular beat engages neurons from medial premotor cortices (MPC), which encode the duration and serial order of the produced intervals, creating population neural

state trajectories that represents the rhythmic tempo in state space. These neural trajectories are the projection of the neural population time-varying activity onto a low dimensional state space using principal component analysis. Here we test the effects of modality and metronome tempo on the kinematics of the neural state trajectories. The single unit activity from hundreds of simultaneously cells in the medial premotor cortex (MPC) was recorded during the performance of a synchronization tapping task (ST), where monkeys tapped in synchrony to an auditory or visual isochronous metronome with a tempo of 450 or 850 ms. The results showed a strong periodic pattern in the MPC neural trajectories, generating loops in the state dynamics for every produced interval in the tapping sequence. Importantly, both the amplitude and the distance from the mean of the MPC neural trajectories varied systematically with the duration of the produced interval. Specifically, the median slit of the monkeys' produced intervals showed that the amplitude and distance of the neural trajectories of the intervals below the 25^{th} percentile were statistically different from the intervals above the 75th percentile. Remarkably, there was a bias towards the auditory modality in the strength of rhythmic tempo encoding by the amplitude and distance neural state trajectories. These finding support the notion that MPC is a code node for the representation of beat entrainment to auditory and visual metronomes.

Disclosures: A. Betancourt-Vera: None. **J.A. Gamez:** None. **G. Mendoza:** None. **H. Merchant:** None.

Poster

245. Time Perception

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 245.08/Y4

Topic: H.01. Animal Cognition and Behavior

Support: MH065561
MH073057

Title: Effect of atomoxetine on interval timing with appetitive distracters

Authors: *A. R. MATTHEWS[1], B. S. BEERS[2], M. BUHUSI[1], C. V. BUHUSI[1];
[1]Interdisciplinary Program in Neurosci., [2]Psychology, Utah State Univ., Logan, UT

Abstract: Noradrenergic agonists are used to improve cognitive functioning, performance, and working memory, particularly for the treatment of patients with attentional disorders. Among the processes impaired by distracters, and whose dysregulation is documented in affective disorders, is the ability to time in the seconds-to-minutes range, i.e., interval timing. Presentations of emotional distracters during a peak-interval timing tasks have resulted in delayed timing behaviors (Matthews et al. 2012, Front Integr Neurosci 6: 111), which may suggest that attentional and working memory resources are diverted away from the primary timing task and

used to process a distracting stimulus, as proposed by the Relative Time Sharing (RTS) model (Buhusi & Meck 2009, Phil Trans R Soc B 364: 1875-1885). Here we aimed to investigate the role of norepinephrine within the prelimbic cortex (PrL), which had been previously identified as a structure that was involved in both interval timing behaviors and the relative sharing of attentional resources that are reallocated following emotional distracter presentations (Matthews et al. 2012). Our results show a distinction between the effects of norepinephrine reuptake on the attentional resources during primary timing tasks and timing during distraction. Atomoxetine significantly decreased the time delay following the presentation of pre-exposed distracters, compared to a control group, $F(2,54)=5.69$, $p<0.01$. Results are discussed in relation to the brain circuits involved norepinephrine, as well as the pharmacological management of affective disorders.

Disclosures: **A.R. Matthews:** None. **B.S. Beers:** None. **M. Buhusi:** None. **C.V. Buhusi:** None.

Poster

245. Time Perception

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 245.09/Y5

Topic: H.01. Animal Cognition and Behavior

Support: NCBS, TIFR
NaMoR, department of biotechnology, Government of India
Optics Grant, department of biotechnology, Government of India

Title: How does the brain associate events that do not co-occur?

Authors: *S. BHATTACHARJEE, K. ANANTHAMURTHY, D. SINGH, U. S. BHALLA; Natl. Ctr. for Biol. Sci., Bangalore, India

Abstract: Decades of work describe the hippocampus as being essential for trace learning. Previous work from our laboratory, using a trace learning protocol of 350ms tone(CS), 350ms trace and 50ms air-puff to the eye(US) established that hippocampal CA1 cell firing becomes reliable and can be arranged in a sequential manner to bridging the time gap between the two stimuli (Modi et al., eLife 2015).
While the co-occurrence of CA1 sequential activity with the formation of trace conditioning is suggestive, the downstream processes and nature of information encoded by this neuronal sequence are unknown. Specifically, it is not clear if the sequential activity trace encodes time, stimulus modality, or both. To investigate this, we trained mice on a trace eyeblink paradigm with two different Conditioned stimuli (sound-CS1 and light-CS2) and the same Unconditioned stimulus (US, air-puff to the eye). Simultaneously with the behaviour, we monitored single-unit activity using using 2-photon calcium imaging on a custom-built microscope. We record

unilaterally from the left dorsal CA1 pyramidal cells of GCaMP6f mice. Our recordings spanned several few weeks during which the animal learnt the different associations, including switching between conditioned stimulus modalities.Initially the animals learnt a single CS1-US or CS2-US association during a given session. Then they underwent sessions with both CS1-US and CS2-US pairing, configured as alternating blocks of 5 stimuli for CS1 and CS2 respectively. The mice reliably developed a well-timed eyeblink in response to each of these CSs.From the calcium recording of trained mice over multiple sessions (each session=80 trials, 40 for each pairing), we found that many cells developed a reliable response to both CS1-US and CS2-US pairings (29±3; mean % of total recorded cells±SEM), while others responded reliably to only one CS-US pairing (9±1 for Light-airpuff and 19±3 for Sound-airpuff; mean % of total recorded cells±SEM). More cells responded reliably to Sound-airpuff pairings than Light-airpuff pairings. These results suggest that, over the course of training, the activity of CA1 neurons converges not only to represent time, but also to encode information about the modality of the CS.

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Poster

245. Time Perception

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 245.10/Y6

Topic: H.01. Animal Cognition and Behavior

Support: Colleges of Arts and Sciences, University of San Diego

Title: Hippocampus lesions impair time discrimination in rats

Authors: *N. S. TABRIZI[1], G. MARSHALL[1], A. MCLAGAN[1], L. LEIJA[1], M. SABARIEGO[2], J. B. HALES[1];
[1]Psychological Sci., Univ. of San Diego, San Diego, CA; [2]Dept. of Neurosci. and Behavior, Mount Holyoke Col., South Hadley, MA

Abstract: Space and time are both essential features of episodic memory, for which the hippocampus is critical. The involvement of the hippocampus in spatial processing was first described following the discovery of neurons, known as place cells, which fire with spatial-specificity (O'Keefe and Dostrovsky, 1971). More recently, the existence of hippocampal neurons, called time cells, were reported that fire at successive moments in temporally structured experiences (MacDonald et al., 2011). While spatial tasks have been used for the study of place cells, the tasks used for the study of time cells have not used time as the manipulated variable, and therefore the behavioral relevance of this cell firing is unclear. In order to directly study the role of the hippocampus in processing elapsed time, we created a novel time duration

discrimination task. Twelve rats were tested on a figure-8-maze and experienced a 10- or 20-second time delay at the end of the center arm. During this delay, a 2000Hz tone played for the 10- or 20-second duration. Rats learned to make a decision to turn left or right out of the delay box depending on the preceding tone duration (10 seconds = left turn; 20 seconds = right turn). Once the rats reached criterion performance of 90% correct on two out of three consecutive days, they received either an excitotoxic hippocampal lesion or a sham lesion surgery. After recovery, rats were tested to determine hippocampal involvement in discriminating time duration. Rats with hippocampal lesions performed at chance-level on their first testing day post-lesion, and they were impaired relative to the sham-lesioned rats. Although the hippocampal-lesioned rats began discriminating at above chance-level, they failed to reach criterion even after 50 days of post-operative testing, whereas, sham-lesioned rats needed half that long to return to criterion performance. Results indicate that rats with hippocampal lesions are significantly impaired at discriminating the duration of elapsed time in order to perform the associated behavioral response.

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Poster

245. Time Perception

Location: Hall A

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Program #/Poster #: 245.11/Y7

Topic: H.01. Animal Cognition and Behavior

Support: NIGMS & NIDA SC1DA 034995 (ARD)
NSF 0633497 (TR)
Jewish Communal Fund (TR)
NYS-Star Early College program (TR)

Title: Modeling interval timing with recurrent neural nets

Authors: *T. RAPHAN[1], **E. DOROKHIN**[1], A. DELAMATER[2];
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Abstract: We studied how recurrent neural nets (RNN), which utilize delayed feedback weights, could model the encoding of time at the supra-second level. Recurrent “Go” and “No-Go” neural processing units with different dynamics were identified whose outputs were summated to generate a pulse that drives a fixed integrator unit. This system was used to model empirical data from rodents performing in an instrumental “peak interval timing” task for Tone and Flash inputs. Reward availability was signaled after different times from stimulus onset during training. Rodent performance was assessed on non-rewarded trials, following training, with each

stimulus tested individually and simultaneously in a stimulus compound. The weights in the Go/No-Go network were trained using experimentally obtained mean distribution of bar press rates across an 80 s period. The rewards for tone and flash were given 5 s and 30 s from stimulus onset, respectively. Different Go/No-Go systems were used for each stimulus, but the weighted output of each fed into a final common recurrent integrator unit, whose weights were unmodifiable. The RNN was implemented and trained in Matlab using the data from non-rewarded trials. The neural net output accurately fit the temporal distribution of tone and flash-initiated bar press data. A "Temporal Averaging" effect was obtained when the flash and tone stimuli were combined. Average auto-correlation functions for the tone, flash and compound responses and cross-correlations between their pairwise combinations confirmed that the peaks and variances of the three response functions significantly differed, with the compound being intermediate between tone and flash but somewhat more similar to flash than tone. Combining tone and flash responses were not superposed as in a linear system. Rather, implementation of nonlinear "saliency functions" that limited the output signal of each stimulus to the final integrator when the other was co-present better fit the data. The model suggests that the brain encodes timing through connection weights of a dynamic RNN. One likely pathway for implementing this RNN is the cortico-striatal-limbic circuit, which has been implicated in the estimation and control of interval timing behavior.

Disclosures: **T. Raphan:** None. **E. Dorokhin:** None. **A. Delamater:** None.

Poster

245. Time Perception

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 245.12/Y8

Topic: H.01. Animal Cognition and Behavior

Support: NIH R01MH068073

Title: Behavioral and neural mechanisms of long-interval timing

Authors: ***B. AKDOGAN**[1], K. R. LIGHT[2], B. K. GERSTEN[1], B. COTTEN[2], E. H. SIMPSON[1,3], P. D. BALSAM[2,1,3];
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Abstract: The ability to anticipate when behaviorally and biologically relevant events will occur is a central problem for animals. Even though there is an abundance of research on the timing of durations in the short-interval (e.g., seconds and minutes) and circadian ranges (approximately 24 hrs), research on how time intervals in the hours range are learned and remembered is limited. To investigate the behavioral and neural mechanisms of long-interval timing, we trained 36

C57BL/6j mice on a fixed-interval (FI) timing procedure in which food reward delivery was contingent upon the first lever press that occurred after a fixed amount of time had elapsed since trial onset. Specifically, all mice were trained to learn time intervals that gradually increased from seconds to minutes to hours. Two separate groups of mice were trained until they were able anticipate food rewards at the end of 60- or 120-min long trials in a stable manner. Our findings showed that the rate of lever pressing in both FI groups increased steadily as a function of time, with a slower trajectory in the FI-120 min group. Furthermore, the rate of increase in response rates scaled proportionately with the two target intervals, indicating that the mice were able to time both durations accurately and adjust the timing of their anticipatory lever presses accordingly. To identify the changes in brain-wide neural activity that drives the timing of 60- and 120-min long intervals, we then divided the mice into three further groups and removed them from the operant chamber 3 minutes into the session, 50% through the session, or at the end of the session. We then processed the brains for c-*fos* expression via immunohistochemical staining of cleared brain tissue using the iDISCO+ protocol (Renier et al., 2016). We have recently optimized the parameters for the visualization of activity-induced c-*fos* expression in whole-mount brains using light sheet fluorescence microscopy, as well as for post-imaging data analysis using the *ClearMap* software (Renier et al., 2016). Results of this study identify the behavioral mechanisms of timing of long event durations, which helps bridge the gap between the investigation of short-interval timing and circadian timing. The findings will also provide insight into the characterization of temporal and spatial dynamics of brain-wide neural activity underlying anticipatory timing behavior.

Disclosures: **B. Akdogan:** None. **K.R. Light:** None. **B.K. Gersten:** None. **B. Cotten:** None. **E.H. Simpson:** None. **P.D. Balsam:** None.

Poster

245. Time Perception

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 245.13/Y9

Topic: H.01. Animal Cognition and Behavior

Support: ANR TimeMemory CE37-0014-02
ANR 11 LABEX 0042

Title: Time discrimination in the macaque: Contrasting striatal to hippocampal activity

Authors: F. ROLANDO[1], J.-R. DUHAMEL[1], V. DOYERE[2], ***S. C. WIRTH**[1];
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Abstract: Time can be represented from sub-seconds to minute scales and underlie a wide range of functions from movement planning to the formation of episodic memory. Given results showing the implication of the striatum for the first and of the hippocampus for the second, we recorded neural activity in these two structures in rhesus macaques while they performed an interval-discrimination task. The beginning of the interval to be timed was signaled by the appearance of a cue. Following the cue, three distinct targets appeared on the screen, in three different spatial locations, after a short, intermediate or long interval. To get a reward, the monkeys had to select via a joystick the correct target depending on the time elapsed since the cue: the bottom target after a short interval, the left target after an intermediate interval and the top target after a long interval. Animals discriminated correctly the different durations, at the sub- and supra-second range. Performance were higher for long and short intervals compared to the intermediate one. Reaction times were shorter after long intervals. These results indicate that the monkeys timed intervals effectively and probabilistically. Similar patterns of response were also observed in human subjects. Preliminary results show time related activity in both the striatum and the hippocampus, with stronger activity related to the reward expectancy in the striatum, while activity in the hippocampus was more delay selective.

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Poster

245. Time Perception

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Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 245.14/Y10

Topic: H.01. Animal Cognition and Behavior

Support: NIH F31NS106737
Alfred P. Sloan Foundation
Kwak-Ferguson Fellowship
NIH R01MH118240

Title: Thalamofrontal circuits in timing behavior

Authors: *B. J. DE CORTE[1], K. HESLIN[2], H. E. HALVERSON[3], K. L. PARKER[4];
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Abstract: Effectively timing decisions and actions in the seconds-to-minutes range is critical for daily functioning and is heavily impaired in a variety of neuropsychiatric and neurodegenerative diseases. Timing recruits a diverse set of brain regions and how these areas interact to generate timed behavior is not well understood. The rodent medial frontal cortex and mediodorsal thalamus play a prominent role in cognitive functioning and are thought to mediate these

processes via reciprocal interactions. Therefore, we asked whether these areas are necessary for timing individually and, if so, whether they interact to generate well timed actions. Specifically, we trained rats on an operant task in which they were presented with one of two cues (tone or light) across different trials. Each cue instructed the rat to make a response after a distinct time interval elapsed, in order to earn a reward (e.g., tone-8s / light-16s). In Experiment 1, we implanted cannulae bilaterally into both the mediodorsal thalamus and medial frontal cortex. Reversibly inactivating either area with Muscimol heavily impaired timing performance. Importantly, inactivating either area produced a highly similar deficit. Specifically, when presented with either cue, rats maintained equivalent response rates, relative to saline infusions; however, these responses lacked temporal organization around the cue's target interval. In Experiment 2, we attempted to demonstrate that these deficits emerge due to impaired communication between the thalamus and frontal cortex. To assess this, we trained rats on the same task, and infused the inhibitory opsin ArchT3.0 into the mediodorsal thalamus. Our data currently suggest that inhibiting thalamic projections from this area to the frontal cortex produces a similar timing deficit to that seen when either area is inactivated individually. Collectively, these findings demonstrate the necessity of the mediodorsal thalamus and medial frontal cortex in timing and suggest that these regions may interact to mediate timing behavior.

Disclosures: **B.J. De Corte:** None. **K. Heslin:** None. **H.E. Halverson:** None. **K.L. Parker:** None.

Poster

245. Time Perception

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 245.15/Y11

Topic: H.01. Animal Cognition and Behavior

Support: Consejo Nacional de Ciencia y Tecnología Grant 236836
Programa de Apoyo a Proyectos de Investigacio´n e Innovacio´n Tecnolo´gica Grant IN202317

Title: Population overlap in neural of duration selectivity determines the scalar property of temporal processing in the primate frontal lobe and the basal ganglia

Authors: *O. PÉREZ[1], H. MERCHANT[2];
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Abstract: Temporal information processing is critical for many complex behaviors including speech and music cognition. A principal feature described for interval timing is the Scalar Property that states that variability of interval estimates increases as a function of interval

duration. However, the neural mechanisms behind this property are not known. Previously, we found that neurons in the Supplementary Motor Area (SMA), Prefrontal Cortex (PFC) and Putamen of monkeys are tuned to both the duration and the serial order of the rhythmically produced intervals during a synchronization-continuation tapping task (SCT). Thus, the purpose of this study was to determine whether the scalar property is related to the population representation of duration in this neural circuit. We used principal component analysis to reduce the high dimensional patterns of population activity during task performance. The first three principal components were used as the neuronal population response (>40% variability explained) and a 3D normal distribution was fitted for each interval duration. We found that these distributions overlapped between interval durations, and this overlap increased as a function of interval duration, with a significant correlation with the variability of the monkeys' interval estimates. Notably, this neural correlate of the scalar property was more robust in SMA than in PFC and Putamen. Therefore, these finding suggest that overlap of the neural population representation of duration may determine the scalar property and that temporal information processing is hierarchical, with SMA acting as a core timing structure.

Disclosures: O. Pérez: None. **H. Merchant:** None.

Poster

246. Cognitive Aging I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 246.01/Y12

Topic: H.02. Human Cognition and Behavior

Support: McKnight Brain Research Foundation
NIA AG019610
State of Arizona and Arizona DHS

Title: Relation of daily activity patterns to cortical gray matter maps in the healthy oldest old: Findings from the McKnight Brain Aging Registry

Authors: D. A. RAICHLEN[1], P. K. BHARADWAJ[4], M. K. FRANCHETTI[4], S. A. SIMS[6], R. F. REZAEI[8,9], S. S. MERRITT[10], C. J. JESSUP[4], E. C. PORGES[8,9], D. GELDMACHER[7], G. A. HISHAW[2], N. ALPERIN[11], T. P. TROUARD[3], V. G. WADLEY[12], B. E. LEVIN[10], A. J. WOODS[8,9], T. RUNDEK[10], K. M. VISSCHER[6], R. A. COHEN[8], ***G. E. ALEXANDER**[4,5]; [1]Anthrop., [2]Neurol., [3]Biomed. Engin., Univ. of Arizona Evelyn F. McKnight Brain Inst., Tucson, AZ; [4]Psychology, [5]Psychiatry, Univ. of Arizona and Evelyn F. McKnight Brain Inst., Tucson, AZ; [6]Neurobio., [7]Neurol., Univ. of Alabama At Birmingham, Birmingham, AL; [8]Clin. and Hlth. Psychology, [9]Col. of Publ. Hlth. and Hlth. Professions and Ctr. for Cognitive Aging and Memory, Univ. of Florida and Evelyn F. and William L. McKnight Brain Inst., Gainesville, FL;

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Abstract: Engaging in increasing levels of physical daily activity (PA), while having good sleep quality may help in maintaining cognitive and brain health during aging. Wrist-worn accelerometers provide a way to measure engagement in different aspects of daily activity, including levels of moderate to vigorous physical activity (MVPA), fractal patterns of consistent PA (FPA), as well as movement during sleep, reflecting sleep efficiency (SE). How these different measures of activity relate to brain health in oldest old adults has yet to be investigated. We sought to determine whether having high levels of MVPA, FPA, and SE are associated with greater cortical gray matter in a cohort of oldest-old adults from the McKnight Brain Aging Registry. For this analysis, 64 community-dwelling, cognitively unimpaired older adults, ages 85 to 99 were included [mean±sd age = 87.9±3.3; M/F = 31/33; mean±sd Mini-Mental State Exam = 28.4±1.3]. Volumetric T1-weighted 3T MRI scans were acquired across the McKnight Brain Institutes at the University of Arizona, University of Alabama at Birmingham, University of Miami, and University of Florida - Gainesville. The MRI scans were processed using Freesurfer (v6.0) and total intracranial volume (TIV) was computed using SPM12 to adjust vertex-wise volume maps for head-size. Measures of MVPA, FPA, and SE were acquired with Actigraph accelerometers worn on the non-dominant wrist for up to seven consecutive days. MVPA, FPA, and SE were defined with standard algorithms using the GGIR package (v1.6.0) in R (v3.4.4). Analyses tested the relation of MVPA, FPA, and SE to cortical maps of thickness, area, and volume using extent thresholds to maintain an overall $p<0.05$ false positive rate (Greve & Fischl, 2018). Results showed that, after adjusting for TIV, higher levels of MVPA were significantly associated with increased volumes in the vicinity of left lateral temporal and medial frontal regions. Greater MVPA was also significantly associated with greater cortical thickness in parieto-occipital regions; greater FPA was associated with greater thickness in precentral and inferior parietal regions; and greater SE was associated with increased cortical area in inferior parietal regions. The regions of cortical volume, area, and thickness were also significantly associated with better cognitive performance. Among cognitively unimpaired oldest old adults, engaging in more MVPA and FPA, and having better SE are each associated with enhanced gray matter, involving brain regions that are related to better cognitive functions. Together these results support the benefits of PA and sleep quality for brain health in the context of successful cognitive aging.

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Poster

246. Cognitive Aging I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 246.02/Y13

Topic: H.02. Human Cognition and Behavior

Support: Evelyn F. McKnight Foundation

Title: Functional connectivity in the healthy oldest old: Findings from the McKnight brain aging registry

Authors: *K. M. VISSCHER[1], P. STEWART[2], S. A. SIMS[4], P. K. BHARADWAJ[5], M. FRANCHETTI[6], R. F. REZAEI[9], S. MERRITT[11], C. JESSUP[7], D. A. RAICHLEN[8], E. C. PORGES[10], D. GELDMACHER[3], G. A. HISHAW[7], T. P. TROUARD[7], N. ALPERIN[11], V. G. WADLEY[1], B. E. LEVIN[11], A. J. WOODS[10], T. RUNDEK[11], R. A. COHEN[9], G. E. ALEXANDER[6];
[2]Neurobio., [3]Neurol., [1]Univ. of Alabama, Birmingham, Birmingham, AL; [4]Neurobio., Univ. of Alabama At Birmingham, Birmingham, AL; [5]Psychology, [6]Dept. of Psychology, [8]Anthrop., [7]Univ. of Arizona, Tucson, AZ; [10]Clin. and Hlth. Psychology, [9]Univ. of Florida, Gainesville, FL; [11]Univ. of Miami, Miami, FL

Abstract: Measuring relationships among brain regions by using functional connectivity metrics has been a successful biomarker of disease, and has been shown to relate to cognitive function. The vast majority of this work has been performed in younger adults, and older populations with mean age well under 85. Little work has described functional connections in the oldest old. There are two main benefits of characterizing functional connectivity in healthy oldest old individuals. First, it allows us to characterize what a healthy oldest old brain should look like, identifying typical distributions of functional connectivity metrics in the context of successful cognitive aging. Second, because these oldest old participants have many decades of divergent life experiences and relatively large variability on cognitive metrics, we can examine how variability in cognitive metrics relates to functional connections.
Data were acquired as part of the McKnight Brain Aging Registry, using methods harmonized across four sites at the University of Arizona, University of Alabama at Birmingham, University of Miami, and University of Florida. For this analysis, 62 community-dwelling, cognitively unimpaired older adults, ages 85 to 99 were included who had undergone Volumetric T1-weighted 3T MRI scans and 10 minutes of BOLD resting state data acquisition (TR = 3s) and for whom at least 100 timepoints met our strict quality control parameters. Cortical surfaces for each participant were determined using Freesurfer software (v6.0), and BOLD scans were pre-processed using Ciftify algorithms. All functional connectivity analyses were performed on the individual's cortical surface. Functional connectivity was measured within three well-

characterized networks: Default Mode Network, Cingulo-Opercular Network, and Fronto-Parietal Network.
We found that this cohort of healthy oldest old participants showed strong, reproducible connectivity networks for the three standard networks we tested. Further, level of connectivity within the frontoparietal network was positively associated with scores on the MOCA, consistent with a contribution of cortical network integrity to performance on this test of generalized cognition.
This work shows feasibility for examining connectivity in the healthy oldest old and helps set the stage for understanding how individual variability in connectivity relates to cognitive performance in this oldest old cohort.

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Poster

246. Cognitive Aging I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 246.03/Y14

Topic: H.02. Human Cognition and Behavior

Support: Portage Health Foundation Grant 2015-025

Title: The effect of low-intensity eccentric exercise on motor learning and emotional processing in aging: A preliminary study

Authors: *L. RAJESHKUMAR[1], C. B. MAANIKA[4], J. J. DUROCHER[2], S. J. ELMER[3], K. M. TREWARTHA[1];
[1]Cognitive and Learning Sci., [2]Biol. Sci., [3]Integrative Physiol., Michigan Technological Univ., Houghton, MI; [4]Physical Therapy, Central Michigan Univ., Mount Pleasant, MI

Abstract: Exercise intervention studies have shown that as little as 12 weeks of exercise can lead to improvements in both physical fitness and cognitive function in older adults, particularly executive control. It is unclear whether those improvements translate to improvements in other domains that rely on executive control, like motor skill learning and emotional processing. Additionally, the factors that might underlie individual differences in the extent to which older adults improve executive function and physical fitness are unclear. For this study, we recruited 22 healthy adults (65-85 years of age) to be randomly assigned either to a non-exercise control group, or to an exercise intervention group that performed 12 weeks of low to moderate intensity

eccentric leg exercise (Eccentron). Our aim was to assess if this kind of exercise would lead to improvements in executive control, emotional processing abilities, and a reduced susceptibility to interference in motor learning. Corresponding neurophysiological measures were also assessed using EEG. We collected initial baseline measures of executive control, emotional processing and motor learning (using a visuomotor rotation task) while also recording EEG data. All participants returned 12 weeks later to complete the same tasks again, with the exception that the motor learning task was performed under the opposite visuomotor rotation experienced at baseline. In the motor learning task, we found that the control group experienced more proactive interference from baseline learning to post-test compared to the exercise group. The exercise group also displayed a higher level of emotional processing abilities than controls. Significant correlations were observed between proactive interference and emotional processing abilities. These findings highlight the effectiveness of this type of exercise for improving motor learning and emotional processing skills. They also provide preliminary evidence that the cognitive benefits of exercise for older adults can be extended to domains outside of, but related to executive control and memory with applications in education, training, mental health and rehabilitation.

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Poster

246. Cognitive Aging I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 246.04/Y15

Topic: H.02. Human Cognition and Behavior

Support: McKnight Brain Research Foundation (MBRF)
Center for Cognitive Aging & Memory (CAM)

Title: Age related decreases in cortical GABA concentrations assessed over the lifespan abate in cognitively intact adults over 85 years of age

Authors: *E. C. PORGES[1], G. JENSEN[2], P. K. BHARADWAJ[3], M. FRANCHETTI[3], S. A. SIMS[8], R. REZAEI[1], M. FORBES[1], S. MERRITT[9], C. JESSUP[3], D. A. RAICHLEN[4], D. GELDMACHER[11], G. HISHAW[5], N. ALPERIN[10], T. TROUARD[6], V. WADLEY[12], B. LEVIN[9], A. J. WOODS[1], T. RUNDEK[9], K. M. VISSCHER[13], R. COHEN[1], G. E. ALEXANDER[7], B. FOSTER[1], N. PUTS[14];
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Abstract: Edited magnetic resonance spectroscopy (MRS) allows for non-invasive measurement of GABA, the principal inhibitory neurotransmitter. Prior work has demonstrated an age-associated decrease of cortical GABA concentrations during adulthood. In childhood developmental cohorts an age-related increase has been reported, and in midlife no association was found. Further, no study of cortical GABA levels across the lifespan has been reported. Additionally, it is unknown if these previously reported age-related decreases in cortical GABA continue in the cognitively intact oldest old (85 years and older). To generate a more complete understanding of the lifespan trajectory of cortical GABA including the oldest old, we employed metanalytic techniques to extract data points from all published reports of edited MRS of GABA in human cortex. Analyses were limited to publications where figures contained either water or creatine ratios of GABA, and the age of participants with individual data points present. Data were extracted using WebPlotDigitizer from five peer reviewed manuscripts that met these requirements. In addition, two datasets were contributed by collaborators on this project from their previous data collections, and one new data set collected in the oldest-old (n=55 community-dwelling, cognitively unimpaired older adults, ages 85 to 99 [mean ± sd age = 88.1±3.2; M/F = 18/35; mean ± sd MoCA = 25.1±2.4]). These oldest old participants were recruited as part of the McKnight Brain Aging Registry, and MRS of GABA was collected in a midline-frontal lobe voxel (27cm3) on 3T MRIs across the four McKnight Brain Institutes. The 8 datasets were merged using a Bayesian approach. Each data set was given its own scaling factor, and these parameters were estimated simultaneously with regression parameters using the Stan programming language.

The merged dataset provides evidence for a lifespan trajectory of cortical GABA that is consistent with Log-normal distribution. Of note, when those 85 and older are explored independently from the aggregated lifespan data, there was no age-related decrease. When incorporated into the lifespan model, the oldest old are well predicted by the gradual attenuation characteristic of the far-right tail of the log-normal model. The compiled data, comprised of 739 participants between 8-99 years, provide the first illustration of cortical GABA concentrations throughout the lifespan. Taken together these data present a coherent log-normal shape with an early life increase, mid-life stability, decrease during aging, and an abatement of this decrease in those over 85 who remain cognitively intact.

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Poster

246. Cognitive Aging I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 246.05/Y16

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R37AG025667

Title: A new approach to studying age differences in white matter using a multimodal symmetric voxel-wise fusion of three MRI data types

Authors: *A. MENDEZ COLMENARES[1], H. WANG[2], V. CALHOUN[4,5], E. MCAULEY[6], A. F. KRAMER[7,6], A. BURZYNSKA[3,1];
[1]Molecular, Cell. and Integrative Neurosciences, [2]Statistics, [3]Human Develop. and Family Studies, Colorado State Univ., Fort Collins, CO; [4]The Mind Res. Network, Alberquerque, NM; [5]Psychology and Neurosci., Georgia State Univ., Atlanta, GA; [6]Univ. of Illinois at Urbana-Champaign, Urbana, IL; [7]Northeastern Univ., Boston, MA

Abstract: Deterioration of white matter (WM) is one of the age-related changes in brain health. However, human WM aging has been studied predominantly using a single technique, diffusion tensor imaging (DTI). DTI model is non-specific to any histological mechanism and no single MRI measure can fully capture WM health. Therefore, we propose a novel multimodal approach with data-driven fusion analysis to leverage multiple WM imaging modalities in a joint analysis to study aging effects. We collected T1-weighted images (WI) (MPRAGE, 0.9mm3), T2-WI (FLAIR, 1.7 × 1.7 mm2 in-plane resolution) and DTI (30 diff. dir., b-value=1000s/mm2, TR/TE = 5,500/98 ms, dir b=0) on a 3T Siemens Trio (Erlangen, Germany) from 247 cognitively healthy (MMSE>26) older adults 60-79 years old (NCT01472744). To study age differences in WM, we compared “younger” old (n=64, 60-63y, M=62, 66% female) with “older” old (n=34, 70-79y, M=72, 74% female). Images were processed with FSL, with removal of non-brain tissue and combined linear and non-linear normalization to the MNI template (1mm3 for DTI, 2mm3 for T1- and FLAIR). Analyses were restricted to core regions of WM by using a WM mask (FMRIB58_FA at 47% intensity). For fusion analyses, we used a multiset canonical correlation and joint independent component model (mCCA + jICA) (http://mialab.mrn.org/software/fit) on four mean-centered feature maps: T1-WI, T2-WI , fractional anisotropy (FA) and mean diffusivity (MD). We obtained one cross-modality independent component (IC) that was comprised of age differences in FA and FLAIR. FA-IC was lower in older adults in the genu and body of corpus callosum and the fornix, and regions of higher FA in posterior periventricular regions and optical radiations. FLAIR-IC was increased in older-old adults in posterior periventricular region and parts of body corpus callosum and decreased in the splenium. Aging is known to be related to decreases in FA and increases in FLAIR signal, known as white matter

hyperintensities. The fusion analysis replicated these findings in the expected regions (FA decreases in prefrontal WM and FLAIR-signal increases in posterior periventricular WM). However, we also detected some unexpected age differences (increase in FA and decrease in FLAIR), suggesting that this data-driven approach can help reveal new patterns in the data. Because raw T1 and FLAIR are not quantitative, current results need to be treated as preliminary and will be extended to better measures.

Disclosures: A. Mendez Colmenares: None. **H. Wang:** None. **V. Calhoun:** None. **E. McAuley:** None. **A.F. Kramer:** None. **A. Burzynska:** None.

Poster

246. Cognitive Aging I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 246.06/Y17

Topic: H.02. Human Cognition and Behavior

Title: Differences in motor inhibition of aging musicians and non-musicians

Authors: *E. L. STEGEMOLLER[1], P. IZBICKI[2], A. F. ZAMAN[3];
[2]Neurosci. and Kinesiology, [1]Iowa State Univ., Ames, IA; [3]Kinesiology, Iowa State Univ. Dept. of Kinesiology, Ames, IA

Abstract: Older adult musicians have behavioral and neurophysiological enhancements in various motor domains as compared to non-musicians, suggesting that music training may delay the decline in motor inhibition with aging. Yet, motor inhibition has not been studied across the lifespan in musicians and non-musicians. Thus, the purpose of this study was to investigate the behavioral and neurophysiological differences in motor inhibition in aging musicians and non-musicians. Twenty healthy young adult (HYA) musicians and non-musicians and twenty healthy older adult (HOA) musicians and non-musicians were asked to complete a syncopation task (motor inhibition). Participants were asked to perform an index finger flexion-extension movement (i.e., finger tap) in sync with an auditory tone (i.e., synchronized) and between auditory tones (i.e., syncopated) presented at 1 Hz. The forearm, wrist, thumb, and fingers 2-4 were supported with a brace maintaining the forearm in a pronated position with the elbow flexed at 90 degrees. The index finger remained unconstrained to allow for full range of motion without touching a surface. Transcranial magnetic stimulation (Magstim Model 200, Magstim, Whiland, Carmarthenshire) single-pulse and paired-pulse was performed at rest and between synchronized and syncopated finger taps. Accuracy was recorded using a goniometer. Motor evoked potential amplitude was recorded from electromyography (Delsys, Boston, MA, USA) and analyzed in The Motion Monitor (Chicago, IL, USA). A 2 (HYA, HOA) x 2 (musician, non-musician) ANOVA was completed to determine differences and interactions between groups. Preliminary results reveal there may be behavioral and neural correlates of motor inhibition

between musicians and non-musicians in both age groups. At the conclusion of the study, results will demonstrate a clearer understanding of whether music training contributes to greater inhibitory control during the aging process, thus, enhancing health and quality of life in older adults.

Disclosures: E.L. Stegemoller: None. **P. Izbicki:** None. **A.F. Zaman:** None.

Poster

246. Cognitive Aging I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 246.07/Y18

Topic: H.02. Human Cognition and Behavior

Support: Iowa Women of Innovation: Collegian Innovation and Leadership Winner
Iowa State University Helen Easter Graduate Scholarship

Title: Differences in cognitive inhibition of aging musicians and non-musicians

Authors: *P. IZBICKI, K. RUMEL, E. STEGEMOLLER;
Iowa State Univ., Ames, IA

Abstract: Older adults experience a decline in the domains of cognitive inhibitory control. These declines have been implicated in instrumental activities of daily living. However, studies have revealed that older musicians have behavioral and neurophysiological enhancements in various cognitive domains as compared to non-musicians. This suggests that music training may delay the decline in cognitive inhibition with aging. Yet, cognitive inhibition has not been studied across the lifespan in musicians and non-musicians. Thus, the aim of this study was to investigate the behavioral and neurophysiological differences in cognitive inhibition in aging musicians and non-musicians. Twenty healthy young adult (HYA) musicians and non-musicians and twenty healthy older adult (HOA) musicians and non-musicians were recruited for the study. To measure cognitive inhibition**,** the Stroop task was performed while electroencephalography was recorded. Participants were asked to name the color of a word presented in either red, green, yellow, or blue. Three conditions were presented randomly: neutral (infrequent words sol, helot, eft, and abjure presented in different colors), congruent (color of word matches the word), and incongruent (color of the word does not match the word itself). Accuracy and reaction time were recorded using E-Prime 2.0 (Psychology Software Tools, Pittsburgh, PA). P300 amplitude and latency were recorded, processed, and analyzed using ActiveTwo Bio Semi system (BioSemi, Amsterdam, NL) and MATLAB. A 2 (HYA, HOA) x 2 (musician, non-musician) ANOVA was completed to determine differences and interactions between groups. Preliminary results reveal there may be differences in the behavioral and neural correlates of cognitive inhibition between musicians and non-musicians in both age groups. At the conclusion of the study, results will

demonstrate a clearer understanding of whether music training contributes to greater inhibitory control during the aging process, thus, enhancing health and quality of life in older adults.

Disclosures: P. Izbicki: None. **K. Rumel:** None. **E. Stegemoller:** None.

Poster

246. Cognitive Aging I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 246.08/Y19

Topic: H.02. Human Cognition and Behavior

Title: The effect of music listening on cognitive inhibition

Authors: *A. MEYER, C. ELKIN, E. GETTES, E. GUSTAFSON, M. NORMAN, P. IZBICKI, E. STEGEMOLLER;
Iowa State Univ., Ames, IA

Abstract: Previous literature has found varying links between music listening and cognitive performance. Studies have found that upbeat, high-tempo music may play a role in modulating inhibitory control, specifically cognitive inhibition. Other studies suggest that preferred music could also enhance cognitive performance. However, the effects of listening to high-tempo, preferred music on cognitive inhibition have not been studied. Thus, the purpose of this study was to determine if listening to high-tempo, preferred music improves cognitive inhibition in healthy young adults. We hypothesized that listening to high-tempo, preferred music will result in greater accuracy and decreased reaction time (indicating better performance) on the Stroop task as compared to no music and unfamiliar high-tempo music. Thirty healthy young adults (18-35) were asked to name the color of a word presented in either red, green, yellow, or blue. Three conditions of words were presented randomly: neutral (infrequent words sol, helot, eft, and abjure presented in different colors), congruent (color of word matches the word), and incongruent (color of the word does not match the word itself). The music listening order (i.e. no music, high-tempo unfamiliar music, high-tempo preferred music) was also randomized. Accuracy and reaction time were recorded using E-Prime 2.0 (Psychology Software Tools, Pittsburgh, PA). A 2 (congruent minus neutral, incongruent minus neutral) x 3 (no music, high-tempo unfamiliar music, high-tempo preferred music) repeated measures analysis of variation was completed. Results revealed a significant main effect for the Stroop condition. No main effect of music condition and no interaction effect were found. These findings suggest that neither high-tempo unfamiliar music nor high-tempo, preferred music affect cognitive inhibition. Future studies will examine whether music experience plays a role in Stroop performance as well as whether healthy older adults exhibit similar results.

Disclosures: **A. Meyer:** None. **C. Elkin:** None. **E. Gettes:** None. **E. Gustafson:** None. **M. Norman:** None. **P. Izbicki:** None. **E. Stegemoller:** None.

Poster

246. Cognitive Aging I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 246.09/Y20

Topic: H.02. Human Cognition and Behavior

Support: University of Newcastle, Australia
Hunter Medical Research Institute (HMRI)

Title: Altered cholesterol homeostasis in the aging CNS- A contributor to age-related CNS functional decline

Authors: *E. T. CRESSWELL, M. J. CUMMINS, D. W. SMITH;
Biomed. Sci. and Pharm., Univ. of Newcastle, Callaghan, Australia

Abstract: Aging of the central nervous system (CNS) is associated with functional decline, including cognitive impairment and dementia. Unfortunately, the mechanisms that bring about these age-dependant declines remain poorly understood. The current study expands on an array of recent data indicating that aging impacts the regulation of CNS cholesterol homeostasis. We investigated the effects of aging on the expression of cholesterol homeostasis related genes across CNS regions as well as in specific cell types. Cholesterol is an essential CNS lipid with multiple structural and physiological roles. However, excess free cholesterol is cytotoxic and, therefore, regulation is critical for healthy CNS function. However, this stringent homeostatic control has been shown to be altered with aging. Our studies sought to expand on how aging impacts CNS cholesterol homeostasis. RNA sequencing studies undertaken by our lab to compare CNS expression profiles of young and old c57 mice, found 34 (50%) of a pre-selected group of 68 cholesterol-related genes, were significantly altered with age in the spinal cord and 9 (~13%) in the cerebral cortex. To expand this study, an exploratory approach was taken to investigate other CNS regions (cortex, hippocampus, corpus collosum, cerebellum, as well as white and grey matter regions of the spinal cord) which were macrodissected from CNS cryosections from young (3-4m/o) and old (24m/o) C57Bl6 male mice (n=8/gp). Regional expression changes were analysed using quantitative PCR to identify age-related changes in cholesterol processing genes. We also assessed cholesterol content, using a total cholesterol assay, of samples similarly macrodissected from these CNS regions. Users were blinded to sample grouping throughout. While we found significant expression changes in all regions for genes involved in cholesterol synthesis, transport and hydroxylation, there was a greater number of genes affected by aging in the highly myelinated regions; the cerebellum and WM spinal cord. The greater number of expression changes in these WM-enriched regions of old mice was

reflected in the total cholesterol measurements that showed a similarly greater magnitude of change with age compared to GM regions. For example cholesterol was increased 2.8 fold with aging in the spinal cord WM compared to a 1.8 fold increase in the equivalent GM. To further investigate the effects of aging on CNS cholesterol homeostasis, cell-specific expressional analysis is currently being carried out. To date, analyses suggest CNS cholesterol homeostasis is markedly impacted by aging and we await the outcomes of the cell type-specific analysis to develop a model for these changes.

Disclosures: E.T. Cresswell: None. **M.J. Cummins:** None. **D.W. Smith:** None.

Poster

246. Cognitive Aging I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 246.10/Y21

Topic: H.02. Human Cognition and Behavior

Support: ESF PhD scholarship (100342331)

Title: Hemodynamic activity while driving and conducting secondary tasks in younger and older adults

Authors: *R. STOJAN, C. VOELCKER-REHAGE;
Inst. of Human Movement Sci. and Hlth., Chemnitz Univ. of Technol., Chemnitz, Germany

Abstract: Driving is a complex cognitive-motor task that requires the continuous integration of multisensory information. With increasing age, driving becomes more challenging as a result of reduced cognitive-motor performance. Potential distractions, such as traffic news or manual actions (e.g., adjusting the radio), may additionally challenge brain resources leading to slower reaction times in complex and unexpected situations. In this study, we compared driving performance and brain activity between younger (YA) and older adults (OA) while driving and performing different ecologically valid secondary tasks. Thirty-seven YA (21.8 ± 1.7y, 18 female) and 37 OA (69.6 ± 3.6y, 13 female) regular drivers drove along a rural road (25-30 min) in a driving simulator and performed different secondary tasks: typing a 3-digit number (manual task), comparing traffic news and gas station prices (memory), and stating arguments (reasoning). Lateral car position and velocity was recorded at 10Hz. Mean and standard deviation was calculated for the first 10s after stimulus onset and at baseline driving (no task present). Brain hemodynamic activity was measured using functional near-infrared spectroscopy (fNIRS) over the dorsolateral prefrontal cortex (18 channels) and averaged for 5 to 15s after stimulus onset. For all behavioral data, we found significant main effects of age and condition, with OA driving slower and more variable than YA and differing more between conditions. Furthermore, we found significant interaction effects on all outcomes of interest. Particularly

during the typing task, OA varied considerably more than YA in their lateral car position. For fNIRS, we found no main effect of age, but for condition and a significant interaction effect. OA displayed a distinct increase in prefrontal brain activity during the typing task compared to YA. YA, on the other hand, showed an increase during the argumentation task that was less pronounced in OA. Our results show age-related and task-dependent differences in behavioral driving performance and brain activation during secondary task performance while driving. Particularly when visually distracted, OA seem to have serious difficulties maintaining lateral car position increasing their susceptibility to fatal accidents.

Disclosures: R. Stojan: None. **C. Voelcker-Rehage:** None.

Poster

246. Cognitive Aging I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 246.11/Y22

Topic: H.02. Human Cognition and Behavior

Support: NBRC CORE
(DBT/RLF/Re-entry/07/2014)
DST-CSRI (SR/CSRI/21/2016).

Title: Role of thalamus in reorganisation of cortical hubs with aging

Authors: *M. DAS, A. BANERJEE, D. ROY;
Natl. Brain Res. Ctr., GURGAON, India

Abstract: p { margin-bottom: 0.25cm; line-height: 120%; } The human brain undergoes both structural and functional changes across the lifespan. It is important to know the dynamics of these changes. On average, functional connections within resting-state networks weaken in magnitude while connections between resting-state networks tend to increase with age. A very recent study by Tsvetanov et.al(2016) shows that effective connectivity within and between large scale functional networks changes over the healthy lifespan. We move one step forward to investigate the effect of thalamus in context of healthy aging.
p { margin-bottom: 0.25cm; line-height: 120%;RS fMRI as well as corresponding diffusion weighted (dw) MRI data were collected for 25 young and 24 elderly individuals. Desikan Killiany atlas was used to parcallate the data and various networks measures were used to identify the three resting state networks, Default Mode, Salience, Central Executive. Multivariate GCA is performed to test for causality index between ROIs with and without the thalamus . We have also calculated the distribution of weighted net granger causal outflow with the 100 bootstrap sample of Granger Causality matrix. We have performed nonparametric Mann-whitney U test to test Whether there is any significance difference between net causal outflow for two

different age groups with and without the thalamus.
p { margin-bottom: 0.25cm; line-height: 120%; } Our study shows age-related reorganization in resting state networks. Within network connectivity between the different nodes of three key neuro cognitive networks decreases with age, while, between network functional connectivity increases with age providing evidence either compensatory mechanisms or unspecific increase in neural noise in the aging brain. Salience network plays the role of a mediator as a switch between the default mode network and central executive network. These roles are getting more prominent in the aging brain which is demonstrated by their net causal outflow and causal connectivity. As per our knowledge, our study is the first study to evaluate the role of thalamus in modulating the within and between resting state networks connectivity. This study provides evidences that Thalamus plays a crucial role in modulating the network dynamics and causal flow of information among the resting state cortical networks. Taken together, these results indicate alternation of thalamo-cortical connectivity and causality with aging significantly drives the reorganization in brain networks.

Disclosures: **M. Das:** None. **A. Banerjee:** None. **D. Roy:** None.

Poster

246. Cognitive Aging I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 246.12/Y23

Topic: H.02. Human Cognition and Behavior

Support: University of Minnesota's Kunin Professorship in Women's Healthy Brain Aging to Lisa M. James

Title: Cross-sectional and longitudinal assessment of cognitive function in healthy women

Authors: *S. DOLAN[1], L. JAMES[2], A. P. GEORGOPOULOS[3];
[1]Minneapolis VA Hlth. Care Syst., Minneapolis, MN; [2]Brain Sci. Ctr., Univ. of Minnesota/Minneapolis VAHCS, Minneapolis, MN; [3]Neurosci, Univ. Minnesota, Minneapolis, MN

Abstract: We assessed cognitive function in cognitively healthy women. Participants (N = 87) were enrolled at age 33-96 y and were assessed annually (up to 7 years currently) using the Montreal Cognitive Assessment (MoCA) test. At enrollment, all had a MoCA score of 25 or greater. We found the following. (a) Cross-sectionally, there was a statistically significant reduction in the MoCA score with age at the time of enrollment (first assessment). (b) Longitudinally, a reduction in the MoCA score was observed over the subsequent annual assessments. However, such a reduction was statistically significant only for the 5-7th decades (at first visit). These results indicate that people at middle age are most vulnerable to cognitive

decline. (c) In addition to the cognitive assessment, participants underwent a 1-min resting-state magnetoencephalographic (MEG) scan using a 248 axial gradiometer system (Magnes 3600WH, 4-D Neuroimaging, San Diego, CA) and a sampling rate of 1017 Hz. Zero-lag cross-correlations were obtained between all pairs (N = 30,628) of prewhitened MEG time series (Synchronous Neural Interactions, SNI [1]). In a recent study [2] we showed that the standard deviation (SD) of the absolute value of SNIs increases with age. Here we found that SD was negatively and significantly correlated with the MoCA score. This finding indicates that overall brain network variability (estimated by SD) underlies overall cognitive function (estimated by MoCA), such that an increase in SD results in a decrease of the MoCA score.

1. Georgopoulos et al. (2007) Synchronous neural interactions assessed by magnetoencephalography: A functional biomarker for brain disorders. J Neural Eng 4:349-355.
2. James et al. (2018) The effects of human leukocyte antigen DRB1*13 and apolipoprotein E on age-related variability of synchronous neural interactions in healthy women. EBioMedicine 35:288-294.

Disclosures: S. Dolan: None. **L. James:** None. **A.P. Georgopoulos:** None.

Poster

246. Cognitive Aging I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 246.13/Y24

Topic: H.02. Human Cognition and Behavior

Support: NJ DOH Grant MH-STT-15-001
NIH Grant R01AG053961

Title: ABCA7 genotype moderates the effects of aerobic fitness and exercise on hippocampus-related cognitive function in healthy older African Americans

Authors: *C. BERG[1,2], N. SINHA[2], M. A. GLUCK[2];
[1]New York Univ., New York, NY; [2]Ctr. for Mol. and Behavioral Neurosci., Rutgers Univ., Newark, NJ

Abstract: The neuroprotective effects of aerobic fitness and exercise on Alzheimer's disease (AD) biomarkers are well established, but few studies have examined the differential benefits based on other AD risk factors. Evidence is limited regarding the potential interactive effects of fitness and genetic risk. In particular, ABCA7, a gene whose functions influence mechanisms that are also affected by fitness, and, is associated with significantly greater AD risk in African Americans. We tested the hypothesis that ABCA7 rs3764650 confers an indirect risk for AD in healthy older African Americans (55 years +) through its interaction with aerobic fitness and exercise via two studies: 1) a cross sectional comparison of the effects of fitness on

hippocampus-related cognitive function in 100 carriers of either the non-risk (TT) or high-risk (GG) genotype, and 2) an interventional study with 56 subjects examining exercise-induced improvements in hippocampus-related cognitive function based on ABCA7 rs3764650 genotype. Hippocampus-related cognitive function was measured through performance on the concurrent discrimination and generalization task which measures generalization following rule learning. We found that ABCA7 modulates the association between aerobic fitness and generalization. No group differences were observed on the standardized measures of cognition function; however, for those with the non-risk genotype, higher levels of aerobic fitness were significantly associated with fewer generalization errors, while high-risk genotype carriers did not show any relationship between aerobic fitness and generalization. Moreover, following an exercise intervention, the non-risk group made significantly fewer errors, while there was no improvement in generalization for the high-risk group. Regardless of genotype, the treatment-as-usual control group exhibited no change in generalization. The concurrent discrimination and generalization task may be uncovering prodromal signs of future decline that standardized measures of cognitive function are not yet sensitive to. Overall, these results suggest that the potential disease-modifying effects of aerobic fitness and exercise on AD-related neuropathology may be limited to carriers of the ABCA7 rs3764650 non-risk genotype.

Disclosures: C. Berg: None. **N. Sinha:** None. **M.A. Gluck:** None.

Poster

246. Cognitive Aging I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 246.14/Y25

Topic: H.02. Human Cognition and Behavior

Title: Analysis of neuropsychological performance of healthy older adults practicing aerobic exercise vs. theater

Authors: *Á. F. VILLALVA-SÁNCHEZ[1], H. SALGADO-CEBALLOS[2], J. BERNAL[1], M. RODRIGUEZ-CAMACHO[1];
[1]UNAM, México, Mexico; [2]Med. Res. Unit Neurol Disease, CMN Siglo XXI, IMSS, Ciudad DE Mexico, Mexico

Abstract: Aim: evaluate and compare neuropsychological performance of healthy older adults who practice aerobic exercise or theater.
Method: 20 healthy older adults, spanish-speakers, right-handed, with corrected vision,10 in Aerobic Group (*AG*) and 10 in Theater Group (*TG*), between 60 and 78 *y*, education M=10.8 *y*. Groups were matched for age, gender, and education. Both groups performed a 60 min training session, three times a week, directed by expert instructors for more than 6 months. Exclusion criteria were: obtaining depressive symptoms (GDS), functional dependence (ADL-S & IADL)

and cognitive alterations (CASI, MMSE). Neuropsychological test was used to assess executive functioning (Stroop, TOL, FDT, Block's Corsi) and learning (RAVLT).
Results: Both groups obtained better scores compared with the standardized norm. The comparison between groups significantly higher in learning, attention, planning and working memory of the AG compared to the TG. No significant differences were found between groups in cognitive flexibility and inhibition.
Conclusions: Several studies indicate that macrostructural cerebral affectations are more evident in dorsolateral prefrontal cortex and temporal lobes in the elderly, considering that in the present study cognitive functions associated with these areas were benefited, it is plausible that these two activities improve or stop cognitive deterioration associated with healthy aging. This suggests that both, aerobic physical exercise and performing theater benefit cognitive functioning of older adults, although aerobic physical exercise could offer greater benefits.

Disclosures: Á.F. Villalva-Sánchez: None. **H. Salgado-Ceballos:** None. **J. Bernal:** None. **M. Rodriguez-Camacho:** None.

Poster

246. Cognitive Aging I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 246.15/DP13/Y26

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: H.02. Human Cognition and Behavior

Support: NIH Grant U01AG062371

Title: Gaze driven video games for cognitive training in older adults

Authors: *L. CHUKOSKIE[1], S. HACKER[2], T. L. SIMMONS[1], A.-M. ENGLER[2], L. HILL[3], J. TOWNSEND[2];
[1]Qualcomm Inst., [2]Dept. of Neurosciences, [3]Dept. Family Med. and Publ. Hlth., UC San Diego, La Jolla, CA

Abstract: By 2030, older adults will outnumber children with 25% of the US population age 65 or older. Quality of life throughout healthy aging, especially preserving cognitive function, is a growing concern. Video game-based cognitive training can improve multiple aspects of cognitive function. Our training games are unique in that they use gaze to train attention which forces a high and consistent level of engagement. These games target specific attentional skills that are affected in typical aging, particularly distraction and response inhibition, and demand fast processing to respond effectively to game events. Because control over distracting stimulation is a foundational skill, we expect to observe improvements in distractability to

generalize to other cognitive and practical skills such as driving safety.
We tested the feasibility and pilot efficacy of our suite of 5 gaze-driven training games with an initial cohort of 30 older adults aged 65-80. Participants played training games at home for 12 weeks with a minimum of 30 minutes per session five times per week. Compliance was monitored by remote data collection and outcome testing at 2, 3 & 6 months. As part of the outcome battery, we assessed attention during distraction in a driving task, inhibitory control in an anti-saccade task, and speed of processing via the useful field of view task. We sought to identify the most reliable outcome assessments at different levels of analysis for a larger randomized clinical trial.
Our clinical trial was based on past research that showed promising long-term effects of cognitive training on older adult independence and resistance to cognitive decline. However, our gaze driven games are unique in their demand for consistent intensity of engagement and availability for home-based training. We seek to use this small feasibility study to fine-tune both the training games and select appropriate outcome measures for a larger trial that can assess the efficacy of our game-based cognitive training in both a healthy aging and mild cognitive impairment groups.

Disclosures: **L. Chukoskie:** None. **S. Hacker:** None. **T.L. Simmons:** None. **A. Engler:** None. **L. Hill:** None. **J. Townsend:** None.

Poster

246. Cognitive Aging I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 246.16/Y27

Topic: H.02. Human Cognition and Behavior

Support: R01AG021055-16S1

Title: Posterior cingulate cortical thickness distinguishes SuperAgers from other cognitively normal individuals in the oldest-old

Authors: *E. N. DOMINGUEZ[1], M. M. CORRADA[1], C. KAWAS[1], S. M. STARK[2], C. E. STARK[3];
[1]UC Irvine, Irvine, CA; [2]Neurobio. & Behavior, Univ. of California, Irvine, Irvine, CA; [3]Univ. of California Irvine, Irvine, CA

Abstract: Several reports have identified a group of elderly "SuperAging" individuals who exhibit cognitive performance comparable to middle aged adults (e.g., Geffen et al. 2015; Harrison et al. 2012). Although aging is typically accompanied by widespread brain atrophy, SuperAgers displayed greater cortical thickness than typical agers in the posterior and caudal anterior cingulate cortex, suggesting that anatomy may play a role in their spared cognition. We sought to evaluate the relationship between cortical thickness and SuperAging in the oldest segment of the population, those 90 years and older. This study consisted of 86 cognitively normal (CN) participants from **The 90+ Study** (mean age=94.5, percentage female=64%). Consistent with previous definitions, SuperAgers (n=22) were CN individuals that achieved scores greater than or equal to the mean of younger adults on the California Verbal Learning Test delayed recall (≥8 on short-form), and scores within the top 25th age-normed percentile on Trails-B (≤ 127 sec). Non-SuperAgers (n=64) were CN individuals who did not fit this criteria. Cortical thickness was calculated from T1-weighted structural MRI images using ANTS. Eight *a priori* regions were chosen based on their involvement in SuperAging or neurodegeneration (cingulate cortex, entorhinal cortex, inferior temporal gyrus, middle temporal gyrus, parahippocampal gyrus, precuneus). To enhance signal to noise for exploratory whole-brain analyses, we used a 200-ROI atlas composed of spatially equal ROIs derived from functional connectivity patterns (Craddock et al., 2012). In our *a priori* analysis, we found greater cortical thickness in the left posterior cingulate in SuperAgers relative to non-SuperAgers (delta=0.25 mm, p=.02; adjusting for age, sex, and education). In the whole-brain analysis, no other regions showed reliable differences in thickness aside from an ROI located in the posterior cingulate (delta=0.28 mm, p=0.005; adjusting for age, sex, and education). Consistent with previous work, cortical thickness, particularly in the cingulate cortex, is associated with exceptional performance in the oldest old. Abnormalities in the posterior cingulate have been implicated in early stages of

Alzheimer's disease, possibly highlighting its importance in normal cognitive functioning in aging (Foster et al., 2004, Leech & Sharp, 2014). These findings help highlight the role of this region and extends its importance to the oldest-old.

Disclosures: E.N. Dominguez: None. **M.M. Corrada:** None. **C. Kawas:** None. **S.M. Stark:** None. **C.E. Stark:** None.

Poster

246. Cognitive Aging I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 246.17/Y28

Topic: H.02. Human Cognition and Behavior

Support: R01 AG047972-01A1

Title: Age related neural-vascular uncoupling impairs modulation of neural resources in response to variations in cognitive demand

Authors: *Y. ZHAO[1], D. H. ABDELKARIM[2], M. P. TURNER[2], K. WEST[3], J. HUTCHISON[4], D. SIVAKOLUNDU[5], G. BATCHALLI MARUTHY[4], B. P. THOMAS[7], H. LU[8], B. P. RYPMA[6];
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Abstract: Age-related decline in the modulation of neural recruitment with increasing cognitive task demand has been observed in many studies using blood-oxygen-level-dependent (BOLD) signal. We measured age-changes in demand-related neural modulation in physiological factors underlying BOLD signal while participants performed a digit-symbol-verification task (DSVT). In the DSVT, participants were presented with a key of multiple digit-symbol pairings simultaneously with a single digit-symbol probe pair for 3000 ms. Participants judged if the probe pair matched one of the key-pairings and responded by pressing a right or left thumb-button. The digit-symbol pairings in the key varied parametrically in set size between 1-, 3-, and 9 items. Utilizing a dual-echo fMRI sequence, participants' BOLD signal and cerebral blood flow (CBF) during the task were simultaneously measured. A CO_2 ingestion procedure enabled the calculation of cerebral metabolic rate of oxygen ($CMRO_2$). After pre-processing, general-linear-modeling was conducted independently for BOLD and CBF to model the task-evoked signal changes. The resulting BOLD and CBF parameter estimates within a-priori defined prefrontal regions-of-interest (ROIs) were used to calculate percent signal change (PSC).

$CMRO_2$ changes were then calculated using the deoxyhemoglobin dilution model. In both age groups, reaction time (RT) increased monotonically with set size increases. Across set size conditions, older adults were slower than younger adults. FMRI results showed that, as set size increased, younger adults demonstrated monotonic increases in BOLD, CBF and $CMRO_2$. Older adults showed discrepancies between these three measures: while BOLD showed monotonic increases, CBF peaked at set size 3, then decreased. $CMRO_2$ demonstrated an inverted *U*-shape pattern as set size increased. These results are consistent with previous results from our laboratory indicating age-related CBF dysregulation in response to cognitive task demand. These results suggest that, age-related impairments in neural-vascular coupling limit older adults' capacity to modulate neural resources in response to variations in task demand.

Disclosures: Y. Zhao: None. **D.H. Abdelkarim:** None. **M.P. Turner:** None. **K. West:** None. **J. Hutchison:** None. **D. Sivakolundu:** None. **G. Batchalli Maruthy:** None. **B.P. Thomas:** None. **H. Lu:** None. **B.P. Rypma:** None.

Poster

246. Cognitive Aging I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 246.18/Y29

Topic: H.02. Human Cognition and Behavior

Support: ESF and Sächsische AufbauBank-Förderbank (Project-Number: 100310502)

Title: The relationship of EEG parameters during rest with mild cognitive impairment in adults 80 years and older

Authors: *S. FROEHLICH, C. VOELCKER-REHAGE, K. MUELLER, D. F. KUTZ; Chemnitz Univ. of Technol., Chemnitz, Germany

Abstract: Background. Older adults with mild cognitive impairment (MCI) are at an increased risk to develop dementia. Being able to discover this at risk population can help to enable interventions to slow down disease progression as well as prepare patients and their relatives for the challenges ahead. Electroencephalogram (EEG) recordings during rest seem to be a promising method as they are cost efficient and non-invasive. It is still unclear which parameters prove to be best suited to detect MCI or even predict dementia. Therefore, the purpose of the study is to examine differences in several EEG rest parameters in older adults (OA) with and without MCI.
Methods. Data of 123 older adults (63 male) without diagnosed neurocognitive disease at the age of 80 to 91 years (M = 82.6, SD = 2.6) were collected. Resting EEG measurements with 32 electrodes (actiCHamp system, Brain Products GmbH, Gilching, Germany) during eyes open (for four minutes) and eyes closed (for two minutes) were carried out in a relaxed sitting posture

in a darkened and quiet room. EEG parameters of interest were absolute theta power (4 - 8 Hz) and absolute alpha power (8 - 12 Hz) during eyes closed in occipital (O1, Oz, O2), left parietal (P3, P7) and right parietal (P4, P8) region. In addition, theta and alpha reactivity, which are defined as the power differences between eyes open and eyes closed, were examined. Cognitive status was assessed using the Montreal Cognitive Assessment (MoCA).
Results. Preliminary results showed in 57 (46.3%) of 123 subjects a MoCA score of 25 or less, which is considered an indication of MCI. Artefact free EEG data was available for 111 subjects. Descriptive results showed that alpha power was smaller and theta power was increased in the MCI group in all three regions of interest. But separate variance analysis for both frequency bands revealed no significant main effect of group for alpha power ($F(1,110) = 0.051$ $p = .82$) or theta power ($F(1,110) = 1.340$, $p = .25$).
Conclusion. Although descriptive results showed the expected direction, we were not able to replicate results of other studies (e.g. Babiloni et al., 2009), which showed significant differences between OA with and without MCI. The mentioned EEG parameters in our sample are not clearly related to MCI. They might still have predictive value for cognitive decline in OA as not all MCI cases will develop dementia.

Disclosures: S. Froehlich: None. **C. Voelcker-Rehage:** None. **K. Mueller:** None. **D.F. Kutz:** None.

Poster

246. Cognitive Aging I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 246.19/Y30

Topic: H.02. Human Cognition and Behavior

Title: Cognitive reserve and neuropsychological performance in healthy aging

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Abstract: Cognitive reserve (CR) explains how individual differences in cognitive processing or neural networks underlying task performance, allow some people to cope better than others, in a context of brain damage or during healthy aging. Some studies reported that adults with higher levels of education or occupational activities had lower risks of developing Alzheimer dementia. Little is known about neuropsychological and electroencephalography (EGG) changes in healthy adults before old age and how CR “proxies”: education, occupation and leisure activities help on coping with brain aging. We aim to compare neuropsychological performance in healthy adults with higher levels of CR, and adults with lower levels of CR. We recruited healthy adults (20

women, 16 men) with a range age: 50-65 y (mean 57, SD 4.8); with no reports of neurological, psyquiatric or metabolical diseases, and no mild cognitive impairment according to CASI test (mean 91.5, SD 4.1); Groups were divided according to the Cognitive Reserve Questionnaire, that measures formal education, occupation, and leisure activities.: (1) high cognitive reserve (hCR) (high-cut point ≥13) (2) low cognitive reserve (lCR) (low-cut point = ≤9). Neuropsychological performance included tests of attention and memory (NEUROPSI); language, verbal fluency and working memory (WM) (PIEN); inhibitory control (Stroop Test); and vocabulary (WAIS IV). Also, quantitative EEG was recorded according with 10-20 International System, in resting state condition. Our results show that two groups obtained scores according to the standardized norm, however hCR group showed better performance in the vocabulary task (t=2;p=0.04) and digit forward span test (t=2.7;p=.01), suggesting that language abilities and attention are better preserved. Contrary to some studies in healthy adults that have shown that attention decreases, our results showed that there may be cognitive functions that preserve among aging as an effect of CR. We are analyzing QEGG spectral measures in order to describe if there are differences in the electrical brain organization in both CR groups. These data may allow us to suggest adults on making changes in their lifestyle in order to improve their levels of CR.

Disclosures: **M.A. Gómez López:** None. **Y. del Río-Portilla:** None. **T. Fernandez-Harmony:** None.

Poster

246. Cognitive Aging I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 246.20/Y31

Topic: H.02. Human Cognition and Behavior

Support: CIHR

Title: Age-related differences in the principal gradient of macroscale cortical organization

Authors: ***M. GIRN**[1], A. J. LOWE[2], A. LOCKROW[2], R. SETTON[2], L. MWILAMBWE-TSHILOBO[2], D. S. MARGULIES[4], B. BERNHARDT[2], G. R. TURNER[5], N. SPRENG[3]; [1]McGill Univ. (MNI), Montreal, QC, Canada; [3]Montreal Neurolog. Inst., [2]McGill Univ., Montreal, QC, Canada; [4]Ctr. Natl. de la Recherche Scientifique (CNRS), Paris, France; [5]York Univ., North York, ON, Canada

Abstract: Neurocognitive aging has been characterized by changes in functional connectivity within and between large-scale brain networks. A recent characterization of cortical functional organization revealed a gradient of connectivity from unimodal sensory regions to transmodal association regions of the default network (Margulies et al. 2016, PNAS). This gradient

represents a functional hierarchy from sensorimotor processing to abstract cognition. Here we apply this approach to characterize neurocognitive aging. Resting-state multi-echo fMRI data from 221 cognitively healthy participants was included: 83 older (mean age=68y), 138 younger (mean age=22y) adults. Fluid cognition was assessed with the NIH toolbox. For fMRI denoising, we employed a multi-echo ICA technique, which has demonstrated greater sensitivity to functional connectivity while removing distant dependent motion confounds. We applied a diffusion map embedding algorithm, a non-linear dimensionality reduction technique, to identify gradients at the subject level. Age group differences in the principal gradient was related to cognition using surface-based linear models. Cortical thickness was used as a vertex-wise covariate in these analyses to control for differences in regional grey matter. We found a contraction of the unimodal-transmodal gradient in older adults. Between-group comparisons revealed that the temporal pole, orbitofrontal cortex, inferior temporal gyrus, medial temporal lobe and ventral visual cortex were further isolated from unimodal cortex with advancing age. Regions of superior temporal and inferior frontal gyri and supplementary motor cortex are less isolated from unimodal cortex in older adults. In addition, we found a significant age interaction with the gradient topology and fluid cognition. Positive associations with fluid cognition were greater in ventral visual cortex for young subjects and greater in posteromedial parietal cortex and the inferior temporal gyrus for older subjects. Gradients of functional connectivity are altered from younger to older adulthood and these changes are associated with age-related cognitive change. Cortical gradients provide a novel lens on the shifting functional network of the brain and a neural marker of neurocognitive aging.

Disclosures: **M. Girn:** None. **A.J. Lowe:** None. **A. Lockrow:** None. **R. Setton:** None. **L. Mwilambwe-Tshilobo:** None. **D.S. Margulies:** None. **B. Bernhardt:** None. **G.R. Turner:** None. **N. Spreng:** None.

Poster

246. Cognitive Aging I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 246.21/Y32

Topic: H.02. Human Cognition and Behavior

Support: NIH R01AG047972

Title: Regional variation in neurovascular coupling changes: Effects of age and task demand

Authors: *M. P. TURNER[1], Y. ZHAO[1], D. H. ABDELKARIM[2], K. WEST[3], J. HUTCHISON[1], B. P. THOMAS[5], H. LU[6], B. P. RYPMA[4];
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Imaging Res. Ctr., Univ. of Texas Southwestern Med. Ctr., Dallas, TX; [6]Johns Hopkins Univ., Baltimore, MD

Abstract: Blood-oxygen-level-dependent signal (BOLD) as measured with functional magnetic resonance imaging (fMRI) underpins neurocognitive aging theories. The validity of interpreting BOLD changes with age as a proxy for underlying age-related neural changes relies upon the assumption that BOLD increases monotonically with increasing task demand in consistent fashion across the brain. This assumption can be tested by disentangling two physiologic factors underlying BOLD: cerebral blood flow (CBF), which quantifies the rate of oxygen delivery to metabolically active neural tissue as measured by cerebral metabolic rate of oxygen ($CMRO_2$). The coupling of these two factors reflects the integrity of the neural-vascular coupling system. Sixteen healthy younger (mean age = 23.6, SD = 3.4, 10 F) and eighteen healthy older (mean age = 58.9, SD = 4.6, 11 F) right-handed adults that had been screened for any potential cardiological, respiratory, pulmonary, or vascular conditions performed block-designed visual and motor tasks while undergoing calibrated fMRI scanning. During the visual task, participants responded via bilateral button-press whenever a fixation cross at center-screen changed in luminance. During stimulation blocks, flickering checkerboards were presented at varying frequencies (2 Hz, 4 Hz, and 8 Hz). During stimulation blocks of the motor task, participants pressed buttons bilaterally in rhythm with an auditory cue (1 Hz, 2 Hz, and 3 Hz). To estimate maximum possible BOLD, participants completed a hypercapnia challenge, in which they breathed room air for 4 minutes and then an isometabolic gas containing 5% CO_2 21% O_2, and 74% N_2 for 6 minutes while being scanned at rest. During all functional scans, BOLD and CBF were collected in separate echoes using a novel pCASL-based sequence (parameters TE1/TE2=11/30 ms, TR = 4 s, 22 6-mm axial slices, no gap, in-plane resolution = 3.4 × 3.4 mm^2). In visual cortex, during visual stimulation, younger adults exhibited monotonic increases in BOLD, CBF, and CBF/$CMRO_2$ with increasing task demand. BOLD, CBF, and CBF/$CMRO_2$ for older adults plateaued as task demand increased. In motor cortex, CBF/$CMRO_2$ increased for younger but remained low for older adults. In both regions, CBF/$CMRO_2$ was lower in older than in younger adults. The lower CBF/$CMRO_2$ ratio observed in older adults supports the hypothesis that neural-vascular coupling is reduced in aging. Regional variability observed in how neural-vascular coupling changes in age also challenges the assumptions on which many neurocognitive aging theories are based.

Disclosures: M.P. Turner: None. **Y. Zhao:** None. **D.H. Abdelkarim:** None. **K. West:** None. **J. Hutchison:** None. **B.P. Thomas:** None. **H. Lu:** None. **B.P. Rypma:** None.

Poster

246. Cognitive Aging I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 246.22/Y33

Topic: H.02. Human Cognition and Behavior

Support: NIH R21 AG056145
DANA Foundation

Title: Playing the video game Minecraft improves hippocampal memory in middle-aged adults

Authors: *G. D. CLEMENSON, C. E. STARK;
Univ. of California Irvine, Irvine, CA

Abstract: The effects of environmental enrichment are well established in animal models. Exposing animals to a larger and more stimulating environment has been shown to enhance brain structure and function, and has a particularly robust impact on the hippocampus. Importantly, this improvement has been observed across the lifespan, from young, to middle-aged, to aged animals. Although it is less clear how environmental enrichment applies to humans, we have previously shown that playing certain types of video games can lead to improvements in hippocampal memory in both younger and older adults. Furthermore, with the use of the popular video game Minecraft, we have been able to show that similar to animal studies, the improvements we observed can be at least partly explained by both the amount of exploration of the world of Minecraft and the amount of engagement. Here we take this video game intervention into a population of middle-aged adults to determine whether enrichment, in the form of video games, can improve or even prevent hippocampal cognitive decline associated with aging. We show that similar to young adults, playing the video game Minecraft (30 minutes/day for 1 month) can lead to improvements in hippocampal memory of middle aged adults. This improvement is related to both the amount of exploration that occurs while playing the video game and the amount of engagement with the video game itself. Together these data suggest that playing certain types of video games may act as a proxy for environmental enrichment in humans throughout several stages of aging.

Disclosures: G.D. Clemenson: None. **C.E. Stark:** None.

Poster

246. Cognitive Aging I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 246.23/Y34

Topic: H.02. Human Cognition and Behavior

Title: Neural dysfunction and alexithymia interact to contribute to subjective cognitive complaints in cognitively intact older adults

Authors: S. A. EVANS, E. R. PAITEL, *K. A. NIELSON;
Psychology, Marquette Univ., Milwaukee, WI

Abstract: Objective and Rationale: Alexithymia increases with age and has recently been implicated in poorer executive functioning (EF) and neural dysfunction that may contribute to memory impairment in elders. Importantly, subtle neural dysfunction may be detectable prior to onset of cognitive decline by intra-individual variability (IIV) in event related potential (ERP) amplitude; greater IIV has been suggested as an index of Alzheimer's disease (AD) neuropathology. Moreover, subjective cognitive complaints (SCC) are considered a prodromal indicator of future cognitive decline. Thus, this study examined whether the influence of alexithymia on SCCs is moderated by EF, and more specifically, by ERP amplitude IIV during an EF task. **Methods:** 45 cognitively-intact elders (M_{age}=77.5, 35 female) completed neuropsychological testing, the Memory Functioning Questionnaire (MFQ, to measure SCCs), and the Toronto Alexithymia Scale-20 (TAS-20). EEG data were collected during EF using a Stop-Signal task (i.e., inhibition). Moderation analyses were performed (PROCESS 3.0), co-varying depression and anxiety, with 1) Trail-making Test B (TMTB, an EF test), TAS-20, and their interaction as predictors of SCCs, 2) P300 ERP amplitude IIV at the Pz electrode, TAS-20, and their interaction as predictors of SCCs, and 3) including both moderators in the same model. **Results:** In Model 1 (R^2=0.37; p=0.001), the TAS-20 X TMTB interaction attained significance (b=-0.03, t(45)=-2.96; p=.005); in Model 2 (R^2=0.40; p<0.001), the interaction of TAS-20 X P300 IIV was significant (b=-.32, t(45)=-3.54; p=.001); in Model 3 (R^2=0.45, p=0.001), P300 IIV (b=-0.29; t(45)=-2.99, p=.005) was a more significant moderator compared to TMTB (b=-0.19; t(45)=-1.76, p=.08). These results demonstrate that EF, whether by cognitive or neural index, moderates the relationship between alexithymia and SCCs, suggesting individuals with greater alexithymia and poorer EF are more likely to endorse SCCs. Moreover, neural dysfunction measured by ERP IIV is a more sensitive index than cognition. **Conclusions:** Alexithymia interacts with EF, both behaviorally and by neural dysfunction to increase endorsement of SCCs. Alexithymia is a potentially substantive risk factor for cognitive decline in older adults.

Disclosures: S.A. Evans: None. **E.R. Paitel:** None. **K.A. Nielson:** None.

Poster

246. Cognitive Aging I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 246.24/Y35

Topic: H.02. Human Cognition and Behavior

Support: NSF-GRFP grant DGE-1745038

Title: Conjoint estimation of age-related changes in cognitive and perceptual phenomena

Authors: *K. L. HEISEY[1], K. XIE[2], J. E. PEELLE[3], D. L. BARBOUR[2];
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Washington Univ., Saint Louis, MO; [3]Dept. of Otolaryngology, Washington Univ. in St. Louis, Saint Louis, MO

Abstract: Age-related decline in hearing ability has been identified as a predictor of future cognitive function and Alzheimer's disease progression. Given that deficits in memory are a clear harbinger of cognitive decline, examining the relationship between memory and hearing ability may provide further insight to age-related changes in health and disease. While meta-analyses reveal that measures of noisy speech comprehension correlate to verbal working memory task performance, a model capable of quantifying individual-level interactions between hearing ability and working memory has yet to be established. Our lab has developed a conjoint active machine learning algorithm to link even loosely related input domains and learn the correlation between multiple stimulus spaces. Conjoint estimation is unique in that observations in one domain simultaneously update the model fit over all input domains. Assessing working memory and noisy speech comprehension with a conjoint estimator enables us to examine the relationship between two of the most predictive measures of cognitive decline. Combining a verbal N-back and speech-in-noise test, we leverage active machine learning methods to optimally select the signal-to-noise ratio and memory load to best explore the input domains. The result is a continuous estimate of verbal working memory capacity, noisy speech comprehension, and the interaction between them with sufficient data to enable individual inference. We have validated the accuracy of this conjoint test in young and older healthy adults by comparing performance on discrete speech-in-noise and N-back tests to the conjoint assessment at similar test parameters. While there are distinct cohort-level differences in reaction time and accuracy, intersubject variability reveals individual differences in how limited cognitive resources are utilized, regardless of age. Individual differences in the allocation of neural resources during tasks with competing cognitive demands may help explain the interplay between age, cognitive function, and hearing ability.

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Poster

246. Cognitive Aging I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 246.25/Y36

Topic: H.02. Human Cognition and Behavior

Title: Rann specific functional connectivity in younger and older adults

Authors: G. ARGIRIS[1], *Y. STERN[1], C. HABECK[2];
[1]Cognitive Neurosience Division, Columbia Univ., New York, NY; [2]Taub Inst., Columbia Univ., New York, NY

Abstract: Previous studies have demonstrated that four latent variables, or reference abilities (RAs), can account for the majority of age-related changes in cognition: these being episodic memory, fluid reasoning, speed of processing, and vocabulary (Habeck et al., 2016; Salthouse & Ferrer-Caja, 2003). It has been shown that each RA can be represented by a unique neural network that not only reflects task activation but correlates with RA-related task performance. Furthermore, this network pattern appears to be "age-invariant", such that neural patterns derived in younger adults can accurately predict those patterns observed in older adults (Habeck et al., 2018). In the current study, we attempt to extend previous findings demonstrating RA-specific voxel activations to functional connectivity derived from the same group of participants and as part of an ongoing study with particular focus on any age-related differences. We collected behavioral and fMRI data from 346 community-dwelling adults between the ages of 20 and 80 on a battery of tests relating to the four RAs (three tests per RA = 12 tests). Functional connectivity values were calculated between a pre-defined set of 264 ROIs (nodes) according to Power et al.'s (2011) parcellation scheme. Participants were divided by group into younger (20-49) and older (50-80) adults and we first sought to identify connections (edges) that highly correlated with an RA-specific indicator variable. Next, we correlated behavioral performance with neural connectivity for each task and selected those edges that demonstrated significant positive correlation across all three tasks relating to each RA. Finally, we looked at the conjunction of significant edges between the two analyses to establish the pattern of connectivity per RA in each group. Results indicated a different subset of significant edges for each RA, with the highest number of significant edges observed for fluid reasoning in both younger and older adults. For fluid reasoning, younger adults notably displayed greater connectivity to fronto-parietal task control and dorsal attentional networks whereas older adults displayed greater connectivity in the default mode network. However, for processing speed, younger adults demonstrated greater within-network default mode connectivity whereas older adults demonstrated greater connectivity to visual system regions. Furthermore, there was little overlap between RA-selective edges between younger and older adults. These findings indicate that younger and older adults recruit different functional networks across RAs despite previous evidence suggesting age invariance at the level of voxel activation.

Disclosures: G. Argiris: None. **Y. Stern:** None. **C. Habeck:** None.

Poster

246. Cognitive Aging I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 246.26/Y37

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant HD060986
Nestle Foundation
National Nature Foundation of China 61673090/81330032

Title: Infant malnutrition predicts brain changes associated with accelerated aging in middle-aged Barbadian adults

Authors: A. G. RABINOWITZ[1], M. JAHANSHAHI[2], J. BOSCH-BAYARD[3,1], L. GALAN-GARCIA[4], A. CALZADO-REYES[5], T. DE LOS A VIRUES ALBA[3], M. BRINGAS VEGA[6], P. VALDES-SOSA[3,6], *J. R. GALLER[7];
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Abstract: Childhood malnutrition can have lifelong effects on cognition and behavior (Martorell, 1999), yet little is known about potential long-term effects on aging (Fong et al, 2018). In the present study, we assessed the effects of childhood malnutrition on qEEG and cognitive outcomes in the same individuals at 5-11 years and again at 45-51 years. We hypothesized that early malnutrition leads to changes in brain structure and function and predicts accelerated aging in middle adulthood. Data were collected as part of an ongoing 45-year longitudinal study of the effects of early malnutrition in Barbados. The sample included individuals with (N=46) and without (N= 53) histories of moderate-severe protein energy malnutrition (PEM) limited to the first year of life. Controls were matched by age, gender and handedness and came from the same classrooms. Participants were of Afro-Caribbean descent and predominantly low-middle class. All had qEEG and neurocognitive tests administered in childhood and middle adulthood (i.e. MOCA, MMSE, WASI). Malnutrition effects, adjusted for childhood ecology, were estimated by mixed model multiple regression analyses. We previously reported reported that the qEEG was strikingly different in PEM participants at age 5-11 (Taboada et al 2018). The present report confirmed similar or more striking effects in middle adulthood. Moreover, PEM individuals were significantly more impaired at 45-51 years than at 5-11 years of age on cognitive tests and the qEEG measures. At 45-51 years, we also found a 3-fold increase in the PEM group relative to controls in the prevalence of mild cognitive impairment (15% to 47.8%) using the standard MoCA cut-off score, and a 2-fold increase (from 11% to 21.7% increase) on the MMSE. Finally, the qEEG at both childhood and adult ages was significantly associated with MOCA and MMSE scores. Child malnutrition is one of the most prevalent public health conditions worldwide and qEEG may serve a useful and cost-effective tool to identify such exposed individuals in underserved communities at risk for cognitive decline. Future studies are needed to explore potential pathways and mechanisms linking early malnutrition to accelerated aging.

Disclosures: **A.G. Rabinowitz:** None. **M. Jahanshahi:** None. **J. Bosch-Bayard:** None. **L. Galan-Garcia:** None. **A. Calzado-Reyes:** None. **T. de los A Virues Alba:** None. **M. Bringas Vega:** None. **P. Valdes-Sosa:** None. **J.R. Galler:** None.

Poster

246. Cognitive Aging I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 246.27/Y38

Topic: H.02. Human Cognition and Behavior

Support: NIA AG032361

Title: Age and sex-related differences in default mode network in healthy middle-aged adults

Authors: *J. K. BLUJUS[1], L. E. KORTHAUER[2], I. DRISCOLL[1];
[1]Psychology, Univ. of Wisconsin-Milwaukee, Milwaukee, WI; [2]Psychiatry and Human Behavior, Alpert Med. Sch. of Brown Univ., Providence, RI

Abstract: Alzheimer's disease (AD), a progressive age-related neurodegenerative disorder, is characterized by a long prodromal period where neurodegenerative changes may be occurring decades prior to the onset of clinical symptoms. Identifying individuals at risk for AD during prodromal stages, prior to substantial damage, may improve treatment efforts. Alterations in functional connectivity of the default mode network (DMN) has been identified as a potential marker of AD. To better understand its utility in early disease identification, it is important to characterize age-related changes in DMN connectivity in cognitively normal adults. Middle age, a likely prodromal stage, has been largely omitted from investigations to date. Moreover, it is critical to understand potential sex differences in DMN connectivity, as the prevalence of AD is higher in women. The current study assessed the interaction between age and sex in functional connectivity of the DMN in healthy, non-demented, middle-aged adults (age 40-60; N=123; 74 women). Participants underwent resting-state functional MRI on a GE Signa 3T scanner. Posterior and anterior subnetworks of the DMN were identified using independent components analysis and connectivity within these subnetworks of the DMN were characterized separately. We found no significant effect of age on functional connectivity of the DMN within this limited age range. We did observe a significant effect of sex on DMN connectivity. Women had higher posterior DMN connectivity bilaterally in the precuneus/posterior cingulate cortex regions and the right parahippocampal gyrus compared to men. Women also had higher anterior DMN connectivity than men in the left anterior cingulate cortex, frontal pole, and inferior frontal gyrus. We also observed a significant age by sex interaction in the posterior DMN; older women had lower and older men had higher connectivity in the precuneus. These findings suggest that in middle age, women show stronger connectivity within the DMN compared to men and that age-related changes within DMN differ by sex. Collectively, our results highlight the importance of

considering sex in future characterizations of age-related changes in DMN across adulthood and its use as an early marker of AD.

Disclosures: J.K. Blujus: None. **L.E. Korthauer:** None. **I. Driscoll:** None.

Poster

246. Cognitive Aging I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 246.28/Y39

Topic: I.06. Computation/ Modeling/ and Simulation

Title: Optimization of lobular electric field distribution in cerebellar transcranial direct current stimulation (ctDCS) for age groups of 18-89 years

Authors: *Z. REZAEE, A. DUTTA;
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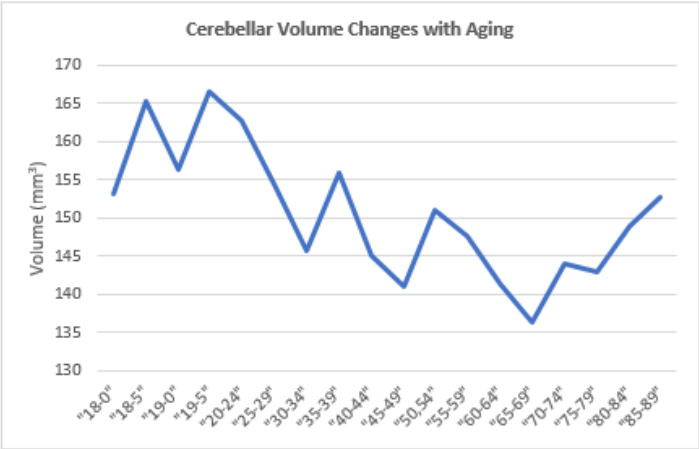
Abstract: INTRODUCTION The one-size-fits-all approach for cerebellar transcranial direct current stimulation (ctDCS) can lead to variability in the cerebellar lobule-specific dosing due to age-related changes in the cerebellar structure. Using our computational pipeline for Cerebellar Lobules' Optimal Stimulation (CLOS)[1], we optimized ctDCS electrode configuration for stimulating superior posterior groups of lobules across ages. This selection was base on previous studies that showed that the superior posterior cerebellum (i.e., lobules Crus I, Crus II, and VIIb) is the most impacted region due to aging [2]. We investigated the effects of cerebellar volume changes on the ctDCS electric field (EF) strength across age groups (18 to 89 years). **METHODS** The specificity in the cerebellar EF strength in superior posterior lobules was investigated across the age groups (Figure1). We assessed the best electrode placement for each age group using sparsity analysis. Then, for each age groups, we optimized EF distribution and electrode configuration for target lobules using our CLOS pipeline. **RESULTS** Specifically, Crus I, Crus II, and VIIb showed a significant change in specificity across age groups that affect ctDCS specificity. Our optimization provided age-specific electrode configuration that improved the ctDCS specificity for the target lobules. **CONCLUSION** The EF dispersion outside of the targeted brain region due to aging [3] requires dose optimization for older adults.
REFERENCES [1] Z. Rezaee and A. Dutta, "Cerebellar Lobules Optimal Stimulation (CLOS): A Computational Pipeline to Optimize Cerebellar Lobule-Specific Electric Field Distribution," *Front. Neurosci.*, vol. 13, 2019. [2]R. Paul *et al.*, "Relative contributions of the cerebellar vermis and prefrontal lobe volumes on cognitive function across the adult lifespan," *Neurobiology of Aging*, vol. 30, no. 3, pp. 457-465, Mar. 2009. [3]S. Mahdavi and F. Towhidkhah, "Computational human head models of tDCS: Influence of brain atrophy on current density distribution," *Brain Stimulation*, vol. 11, no. 1, pp. 104-107, Jan. 2018.



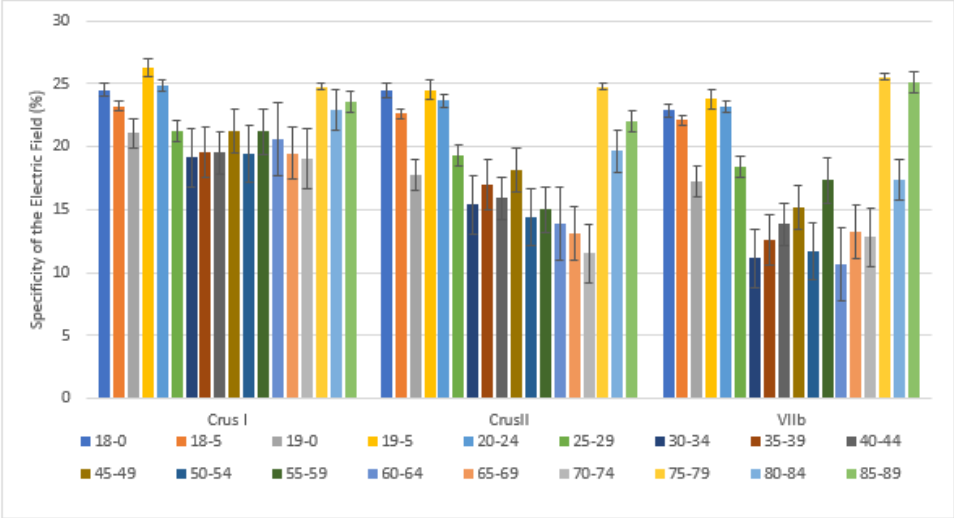


Figure 1-A) Cerebellar volume changes with changes. B) Electric field specificity (eqn. 1) for the cerebellar lobules – Crus I, Crus II, and VIIb – across the eighteen age-group (shown with color). The plot bar shows the Specificity for the specific lobules (Crus I, Crus II, and VIIb). The standard error is also shown on the bar graph.

$$Specificity\ \% = \frac{[\text{Ipsi EF} - \text{Contra EF}]}{[\text{Ipsi EF} + \text{Contra EF}]} \times 100 \quad \textbf{(eqn. 1)}$$

Disclosures: **Z. Rezaee:** None. **A. Dutta:** None.

Poster

247. Cognitive Aging Disorders in Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 247.01/Y40

Topic: H.02. Human Cognition and Behavior

Support: Division of Intramural Research of the National Institute of Nursing Research of the NIH

Title: Cognitive and motor aspects of cancer-related fatigue

Authors: *L. R. FENG, J. REGAN, L. SALIGAN;
NIH, Bethesda, MD

Abstract: Cancer-related fatigue (CRF) is a debilitating symptom frequently reported by patients during and after treatment for cancer. CRF is a multidimensional experience and is often solely assessed by self-report measures. The goal of this study was to examine specific cognitive and physical characteristics that were associated with CRF. A total of 59 subjects with non-metastatic prostate cancer receiving external beam radiation therapy were included in the study. Fatigue was measured by the Functional Assessment of Cancer Therapy-Fatigue (FACT-F). The Stroop Color-Word Interference computerized test and static fatigue test using a handgrip dynamometer were used to assess cognitive and physical characteristics of CRF. FACT-F scores significantly correlated with the Stroop Interference score, but not performance accuracy in the congruent, incongruent, and neutral conditions. Fatigued subjects exhibited decreased in 50% exhaustion time and increased static fatigue index in the handgrip test; whereas, maximal grip strength was not affected. The results suggest that CRF exhibits both cognitive and physical characteristics. Subjective fatigue was associated with increased time required to overcome cognitive interference, but not cognitive performance accuracy. Fatigued patients exhibited decreased physical endurance and the ability to sustain maximal strength over time. These objective measures may serve as valuable tools for clinicians to detect cognitive and physical impairment associated with CRF.

Disclosures: L.R. Feng: None. **J. Regan:** None. **L. Saligan:** None.

Poster

247. Cognitive Aging Disorders in Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 247.02/Y41

Topic: H.02. Human Cognition and Behavior

Title: Detection of tauopathy by measurement of phosphorylated tau protein in cerebrospinal fluid for elderly major psychiatric patients

Authors: *M. TAKEBAYASHI[1,2], **K. ITAGAKI**[3,2], W. OMORI[2], N. KAJITANI[2], M. OKADA-TSUCHIOKA[2], H. ABE[2], A. MACHINO[2];

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Abstract: Background: Major psychiatric disorders among the elderly, including depression and schizophrenia, are risk factors, and a possible prodromal stage, of dementia for conditions such as Alzheimer's disease (AD), which is related to tauopathy. Clinically, elderly psychiatric patients often have a comorbidity of dementia including AD. The measurement of phosphorylated tau protein (p-Tau) in cerebrospinal fluid (CSF) is useful for early detection of AD and is authorized under the National Health Insurance of Japan. Among psychiatrists, however, there is resistance to providing an examination with lumbar puncture (LP). As such, there is the risk that tauopathy, including AD, might not be properly diagnosed due to the absence of p-Tau measurement for elderly psychiatric patients. Thus, the current study aims to identify tauopathy in elderly psychiatric patients by measurement of CSF p-Tau level.
Method: CSF was obtained by LP from 46 elderly psychiatric inpatients (28 mood disorders, 9 schizophrenia and 9 other psychiatric disorders) at our facility from January 2015 to December 2017. All subjects were recommended to receive LP due to mild brain atrophy on head MRI/CT, amnesia, or loss of attention in the differential diagnosis of dementia. The mean age was 71.7 ± 10.2 years old, and the mean Hasegawa Dementia Scale-Revised (HDS-R, normal range: more than 20 points) was 23.6 ± 4.1 points. Subjects had not been given a diagnosis of dementia including AD at the time of the LP. An enzyme-linked immunosorbent assay determined p-Tau levels. After procedures were fully explained, written informed consent was obtained from all subjects. The current study was approved by the Ethics Committee of Kure Medical Center and Chugoku Cancer Center.
Results: In 10 of 46 subjects (5 mood disorders, 3 schizophrenia and 2 other psychiatric disorders), CSF levels of p-Tau exceeded the upper limit of the standard value for AD (normal range < 50 pg/ml). The mean age was 74.8 ± 8.0 years old for the 10 subjects with abnormal p-Tau levels. Mean HDS-R was 23.2 ± 8.01 points, and 7 of 10 subjects had normal HDS-R points. All subjects, including their family members, received the clinical results, and 6 subjects started treatment for dementia. Only one subject had a mild post-LP headache that was quickly resolved with acetaminophen.
Conclusion: In the current study, more than 20% of elderly psychiatric patients had an abnormal p-Tau level upon CSF evaluation, suggesting that a considerable number of them might have tauopathy as a comorbidity. In a safe approach, we were able to detect tauopathy, including for AD, early in the clinical course and started treatment based upon measurement of CSF p-Tau levels.

Disclosures: **M. Takebayashi:** None. **K. Itagaki:** None. **W. Omori:** None. **N. Kajitani:** None. **M. Okada-Tsuchioka:** None. **H. Abe:** None. **A. Machino:** None.

Poster

247. Cognitive Aging Disorders in Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 247.03/Y42

Topic: H.02. Human Cognition and Behavior

Title: Relationship between anxiety and cognition in patients with mental illness

Authors: *K. TAKAI, M. YAMAMOTO, T. SHIOIRI;
Gifu Univ. Grad. Sch. of Med., Gifu, Japan

Abstract: Many patients with mental illness, including those with dementia, experience anxiety at some level, and this is one of the most important factors affecting their prognosis and quality of life. Cognition strongly influences anxiety; conversely, pathological anxiety promotes negative cognition. However, to the best of our knowledge, only few studies have examined the relationship between anxiety and cognition. In this study, we evaluated two aspects of cognition, namely emotional competence (the ability to properly assess and adjust a person's emotions) and intelligence quotient (IQ), and investigated their relationship with anxiety. The study was approved by the Ethics Review Committee of the Graduate School of Medicine, Gifu University Graduate School of Medicine and was conducted in accordance with the Declaration of Helsinki, and informed consent was obtained from all the participants. The study included a total of 56 patients (26 women and 30 men aged 18-91 years, mean 55.62) who visited the psychiatric department at Gifu University Hospital. The levels of anxiety were evaluated on the basis of the State-Trait Anxiety Inventory; emotional competence, the Japanese version of a short form of the Profile of Emotional Competence; and IQ, the Japanese Adult Reading Test. The results mainly showed a significant positive correlation between the IQ and the levels of trait anxiety and a significant positive correlation between the emotional competence and the levels of state-trait anxiety. The patient diagnosed with mild cognition impairment tended to have higher anxiety scores than those with Alzheimer's disease. These findings suggest that patients with high IQ and less control over their emotions experience stronger anxiety, and adequate assessment of anxiety leads to an early diagnosis of dementia. We intend to continue this study with more number of participants to provide clearer evidence.

Disclosures: K. Takai: None. **M. Yamamoto:** None. **T. Shioiri:** None.

Poster

247. Cognitive Aging Disorders in Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 247.04/Y43

Topic: H.02. Human Cognition and Behavior

Support: LSU Discover Undergraduate Research Grant (AC)

Title: Prolonged cognitive deficits in young adults with a history of a concussion from high school

Authors: A. CAFFEY, *M. DALECKI;
Sch. of Kinesiology, Louisiana State Univ., Baton Rouge, LA

Abstract: Sport-related concussions can hinder cognitive functions in older individuals years after an incident. Other studies showed impairments in cognitive processing speed of young adults that were tested 6-8 month's post-injury. However, it is not known whether cognitive impairments transfer into young adulthood when a concussion was sustained even earlier in life. The present confirmatory study therefore investigated cognitive functions in young adults with a history of concussion >8 months post incident.
13 young adult college students (7 females) with a history of concussion (CH) (mean 21 yrs.; mean time post-concussion 48 months, range 10-90 months) and 20 age- and education-matched controls with no-history of concussion (NoH; 11 females) performed two cognitive tests on a laptop. The D2 sustained attention test involved sequences of nine letters (d and p) presented on the screen, and each letter was framed with different numbers of superscript or subscript of commas. Participants had to press a 'D2' button when seeing the letter d surrounded by two commas, and a 'not D2' button otherwise. A new sequence of nine letters appeared once the previous sequence was finished. A block of sequences was terminated after 30 seconds, 12 blocks were presented overall. The Stroop color word test assessed participant's ability to inhibit prepotent responses. Four words were presented on the screen in two test conditions of 48 trials. In a congruent condition, meaning and color of the word coincided, and in an incongruent condition, meaning and color of the word differed. Participants denoted per key press the color they saw. We analyzed response inhibition (Stroop), sustained attention level (D2), and response time and error score (Stroop and D2) between both groups (CH, NoH) using ANOVA.
In the Stroop task, CH participants showed significantly slower response time in the incongruent task condition ($p<0.01$) and a higher error rate in both task conditions than NoH controls (both $p<0.05$). In the D2 test, sustained attention level, response time, and error rate did not differ between both groups (all $p>0.05$). The current findings suggest processing speed and decision-making deficits during a Stroop response inhibition task in young adults tested about 4 years post-concussion. This result may speak for subtle prolonged functional and or structural brain

changes. Further analysis can examine potential correlation between cognitive deficits, time since last concussion, and number of concussions. Future studies could investigate whether prolonged cognitive dysfunctions correlate with brain changes, motor control functions, and whether the deficits transfer into older adulthood.

Disclosures: A. Caffey: None. **M. Dalecki:** None.

Poster

247. Cognitive Aging Disorders in Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 247.05/Y44

Topic: H.02. Human Cognition and Behavior

Title: A novel kit for early diagnosis of Alzheimer's disease using a fluorescent nanoparticle imaging

Authors: *J.-S. PARK[1], S.-T. KIM[2], S.-Y. KIM[2], M.-G. JO[1], M.-J. CHOI[3], M.-O. KIM[1]; [1]Gyeongsang Natl. Univ., Jinju, Korea, Republic of; [2]Seoul Natl. Univ. Bundang Hosp., Seongnam, Korea, Republic of; [3]Res. and Develop. Center, Phytos Inc, Anyang, Korea, Republic of

Abstract: Alzheimer’s disease (AD) is a progressive neurodegenerative disease and chronic illness with long preclinical phases and a long clinical duration. Until recently, a lack of potential therapeutic agents against AD was the primary focus of research, which resulted in less effort directed towards developing useful diagnostic approaches. In this study, we developed a WO2002/088706 kit that is composed of fluorescent nanoparticles for the early detection of AD. We provided a fluorescent nanoparticle for detecting markers and a kit for the early diagnosis of AD. The kit consists of a probe molecule comprising an oligonucleotide capable of detecting one or more AD-specific microRNAs (miRNAs) and biomarkers related to AD. Through screening, we selected miR-106b, miR-146b, miR-181a, miR-200a, miR-34a, miR-124b, miR-153, miR-155, $A\beta_{1-42}$ monomer (mAβ), $A\beta_{1-42}$ oligomer (oAβ), UCHL1, NLRP3, Tau, STAT3, SORL1, Clusterin, APOE3, APOE4, Nogo-A, IL-13, and Visfatin to serve as AD- and inflammation-related markers. For detection of kit-binding properties, we checked the expression levels of amyloid beta (Aβ), tau protein, and inflammatory mediators in APP/PS/ApoE knockdown (KD) mice and a control group using co-localisation analysis conducted with a confocal microscope. Using a similar approach, we checked the expression levels of miRNAs in HT22 cells. Finally, we used the plasma from AD patients to confirm that our fluorescent nanoparticles and the WO2002/088706 kit will provide a possible early diagnosis to serve as an AD detector that can be further improved for future studies on targeting AD.

Disclosures: **J. Park:** None. **S. Kim:** None. **S. Kim:** None. **M. Jo:** None. **M. Choi:** None. **M. Kim:** None.

Poster

247. Cognitive Aging Disorders in Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 247.06/Z1

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant AG017586
NIH Grant AG052943
NIH Grant AG054519
NIH Grant NS101863
Wyncote Foundation
anonymous donor

Title: Longitudinal trajectories of conversational ability in Alzheimer's disease and behavioral variant frontotemporal dementia

Authors: *M. L. HEALEY, M. GROSSMAN;
Neurol., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Previous research has shown that the happiest individuals are those who spend the most hours engaged in conversation with others (Mehl et al., 2010). Unfortunately, many neurodegenerative diseases, including both Alzheimer's disease (AD) and the behavioral variant of frontotemporal dementia (bvFTD), can result in language and communication deficits over the course of the disease. For example, patients with AD may show difficulty in naming and verbal fluency, and patients with bvFTD may show deficits in narrative organization and non-literal language comprehension. Here, we use the Perception of Conversation Index (PCI)- an informant-based measure of conversation difficulties in non-aphasic forms of dementia- to characterize the longitudinal trajectories of conversational ability in AD and bvFTD. Caregivers of 21 AD patients and 21 bvFTD patients completed the PCI at two timepoints, that were ~1.52 years apart on average. Mixed effects models predicting annualized change in PCI indicate a significant interaction of disease duration (i.e. symptomatic years) and clinical phenotype (β=6.45, p=0.01). While conversation difficulties in AD remain stable over time (β_{DisDur}=2.30, p=0.21), conversation difficulties in bvFTD increase as a function of disease duration (β_{DisDur}=9.42, p<0.0001). Importantly, these results are not attributable to differences in caregiver burden (β=0.25, p=0.27) or age of onset (β=-0.47, p=0.48) across groups. Next, we explored the potential cognitive mechanism(s) underlying the effect observed in the bvFTD cohort. Linear models demonstrate that the annualized change in conversation metric is best predicted by baseline executive function (here tested with letter-guided verbal fluency) (β=-1.14, p=0.02,

R^2=0.40). Finally, we examine if patterns of cortical thinning at baseline can predict subsequent decline in conversational ability. Permutation-based regression analyses using T1 structural MRI show that cortical thinning in medial, dorsolateral, and orbital frontal cortices at baseline is associated with faster rates of decline in conversation. Taken together, our results in bvFTD have considerable prognostic value: individuals with poor executive function and focal disease in prefrontal cortex at baseline are most likely to experience communication problems later on in disease. Considering recent efforts to develop behavioral and neuromodulatory interventions for language-impaired populations, these at-risk individuals and their caregivers may be able to initiate effective conversational repair strategies prior to symptom onset and thereby preserve functional communication skills.

Disclosures: M.L. Healey: None. **M. Grossman:** None.

Poster

247. Cognitive Aging Disorders in Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 247.07/Z2

Topic: H.02. Human Cognition and Behavior

Title: Evaluation of cognitive function using neural network analysis before and after revascularization surgery for internal carotid artery stenosis

Authors: *M. KOHTA, Y. FUJIMOTO, Y. YAMAGUCHI, A. FUJITA, E. KOHMURA; Dept. of Neurosurg., Kobe Univ. Grad. Sch. of Med., Kobe, Japan

Abstract: Objectives: Internal carotid artery stenosis (ICS) can lead to cognitive impairment as well as ischemic stroke. Although carotid revascularization surgery, such as carotid endarterectomy (CEA) and carotid artery stenting (CAS), can prevent future strokes, the effect of revascularization on cognitive function is controversial. In recent years, the analysis of functional connectivity (FC) in resting-state functional MRI (rs-fMRI) has been used to investigate the effects of cognitive interventions. In this study, cognitive function is evaluated in ICS patients undergoing revascularization surgery with rs-fMRI. Methods: We prospectively enrolled ICS patients, who were expecting the intervention of CEA or CAS. Cognitive assessment, including the Mini-Mental State Examination (MMSE), the Frontal Assessment Battery (FAB), and the Japanese version of the Montreal Cognitive Assessment (MoCA-J) and rs-fMRI were administered ≤ 1 week preoperatively and postoperatively at 1 year. The FC between seed regions (medial prefrontal cortex (MPFC), lateral parietal (LP), posterior cingulate cortex (PCC)) was compared between ICS group (n = 18) and control group (non-ICS patients, n = 10). Also, in the ICS group, FC was compared before and after procedures. Results: Compared to the control group, the ICS group exhibited weaker FC between LP and paracingulate gyrus. After revascularization surgery, significant improvement in the score of MMSE (27.9 vs 29.2, P =

0.014), FAB (16.1 vs 17.3, P = 0.012), and MoCA-J (23.8 vs 27.6, P < 0.0001) was found. ICS group (n = 18) exhibited increase FC between MPFC and paracingulate gyrus using paired t-tests with Δ-MoCA-J score as a nuisance covariate. Conclusion: Revascularization surgery for ICS improves cognitive function. Increase of connectivity between MPFC and paracingulate gyrus may contribute to the cognitive improvement.

Disclosures: M. Kohta: None. **Y. Fujimoto:** None. **Y. Yamaguchi:** None. **A. Fujita:** None. **E. Kohmura:** None.

Poster

247. Cognitive Aging Disorders in Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 247.08/Z3

Topic: H.02. Human Cognition and Behavior

Support: CONICYT PFCHA/DOCTORADO BECAS CHILE/2016 - 21180413
BNI ICM P09-015-F
Fondecyt 1150736
Fondecyt 1151297
Fondecyt 1190958

Title: Modification of functional connectivity as an early mechanism of impairment in spatial memory in patients with cognitive impairment

Authors: *I. PLAZA-ROSALES[1,4], S. MADARIAGA[4], M. BEHRENS[2,3,5], A. PAULA-LIMA[6,4], P. E. MALDONADO[2,4];
[1]Dept. of Med. Technol., [2]Neurosciences, [3]Neurol. and Neurosurg., Univ. of Chile. Fac. of Med., Santiago, Chile; [4]Biomed. Neurosci. Inst., Santiago, Chile; [5]Ctr. de Investigación Clínica Avanzada, Santiago, Chile; [6]Inst. for Res. in Dent. Sci., Univ. of Chile. Fac. of Dent., Santiago, Chile

Abstract: Alzheimer's disease (AD) is the most common neurodegenerative disorder, causing progressive deterioration of neuronal networks, which induces aberrant patterns of activity in neuronal circuits and Functional Connectivity (FC) alterations. A transition state described as Mild Cognitive Impairment (MCI), presents earlier deficits in the spatial navigation ability as a reflection of the damaged hippocampal network widely reported in AD patients. We hypothesized that long-range FC of networks in a navigational task acts as an early marker of deterioration in AD. We conjectured that alterations of long-range FC constitute part of the early mechanisms of amnestic MCI (aMCI). Using eye movement tracking and brain activity recordings with EEG, we compared a control (n=9) and an aMCI (n=9) group while performing a virtual navigation task. We found that a) There was a significant difference in the electrical

brain activity between groups reflected in the analysis of spectral power and FC as synchrony in low-frequency bands, b) the spatial navigation of the aMCI group is significantly worse than the control group with respect to a greater latency to the platform, a higher error rate and a lower average speed during the search of the hidden platform, c) the amplitude of the fixation evoked potentials in the region of interest (occipital electrodes) was significantly lower in the aMCI than the control group and d) there were no significant differences between groups regarding parameters of ocular behavior (fixations and saccades). Differences in our results are difficult to attribute to learning processes because they involve more brain regions and more processing time. However, they may be a warning of early visual processing difficulties with repercussions on the activation of the cortices involved in the creation of the cognitive map in spatial navigation. We conclude that FC may help to predict progression to AD.
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Disclosures: I. Plaza-Rosales: None. **S. Madariaga:** None. **M. Behrens:** None. **A. Paula-Lima:** None. **P.E. Maldonado:** None.

Poster

247. Cognitive Aging Disorders in Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 247.09/Z4

Topic: H.02. Human Cognition and Behavior

Support: JSPS Grant-in-Aid for Young Scientists (B) 17K17811
JSPS Grant-in-Aid for Young Scientists (B) 25870325

Title: Frontal volume and function predict physical exercise efficacy in healthy elderly people: The Mihama-Kiho scan project

Authors: *K.-I. TABEI[1,2], M. SATOH[2], J.-I. OGAWA[4], T. TOKITA[5], N. NAKAGUCHI[6], K. NAKAO[7,8], H. KIDA[2,9], H. TOMIMOTO[3];
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Abstract: Objective: In older adults, physical exercise with music induces greater positive effects on visuospatial function (Satoh et al., 2014) and leads to more extensive neuroanatomical

changes (Tabei et al., 2017) than exercise alone. However, the neuropsychological factors influencing interventions of physical exercise, as well as the neural basis of its efficacy, remain unknown. We aimed to determine whether neuropsychological deficits and brain atrophy could predict the efficacy of physical exercise interventions. Methods: One-hundred twelve participants of healthy elderly people were monitored for 1 year; 51 underwent an intervention involving physical exercise with music, and 61 performed the physical exercise without music. Participants with an increased MMSE score of 2 points or more were included in the improvement subgroup, while the remaining participants were included in the no-improvement subgroup.Results: The no-improvement subgroup performed worse than the improvement subgroup on the word fluency and visuospatial test at baseline. In the no-improvement subgroup, voxel-based morphometric analysis at baseline revealed more extensive gray matter decrease in the left inferior frontal gyrus and medial frontal gyrus. Conclusions: Our findings suggest that some characteristics of pre-intervention cognitive dysfunction and regional brain atrophy may aid clinicians in determining the physical exercise efficacy in healthy elderly people.

Disclosures: **K. Tabei:** None. **M. Satoh:** None. **J. Ogawa:** None. **T. Tokita:** None. **N. Nakaguchi:** None. **K. Nakao:** None. **H. Kida:** None. **H. Tomimoto:** None.

Poster

247. Cognitive Aging Disorders in Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 247.10/Z5

Topic: H.02. Human Cognition and Behavior

Support: NIDDK Grant K23DK113119-02

Title: White matter integrity and cognitive outcomes in hemodialysis patients

Authors: *W. T. RICHERSON[1], D. F. WOLFGRAM[2], B. D. SCHMIT[1];
[1]Biomed. Engin., Marquette Univ. and Med. Col. of Wisconsin, Milwaukee, WI; [2]Dept. of Med., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Background: The purpose of this study is to understand how white matter health in End Stage Renal Disease (ESRD) patients on hemodialysis (HD) affects cognitive decline and how those affects cause cognitive impairments. There are over 600,000 people with ESRD who require dialysis in the United States; the vast majority of which are treated with HD. Cognitive impairment in these patients has been estimated in the range of 60%-70%. There are numerous factors that could contribute to cognitive impairments, however there is evidence that shows there is a HD specific cause of cognitive decline related to hemodynamic fluctuations during HD. We hypothesize that oximetry obtained during HD and white matter integrity measured by diffusion tensor imaging (DTI) correlate with cognitive performance.

This is part of an ongoing exploratory study, but this abstract will focus on initial results.
Study Methods: We collected demographic data, comorbidities, intradialytic measurements of blood pressure and cerebral oximetry, cognitive measures in several domains using NIH Toolbox Cognition Battery, diffusion weighted and anatomical MRIs for 20 participants on HD. Specific tracts were identified using the Johns Hopkins white matter atlas and used to calculate the average DTI measurements in each tract. Regression analysis was used to examine the relationship between mean DTI measurements of white matter integrity and cognitive performance scores. In addition, we compared diffusion MRI and T1 anatomical images of 16 healthy age matched controls from a previous study.
Results: Thus far we found widespread decreases in DTI white matter integrity compared to healthy age matched controls. Decreased integrity was found in tracts implicated as important for cognition including the forceps minor and cingulum bundle. Regression analysis of mean DTI measures identified significant relationships between the white matter integrity of the cingulum, inferior longitudinal fasciculus and uncinate fasciculus and cognitive performance as well as intradialytic hemodynamic fluctuations.
Conclusions: We found a widespread decrease in white matter integrity and significant correlations between cognitive performance and specific tract integrity in our HD population using regions identified using the Johns Hopkins white matter atlas. This analysis shows that HD patients have decreased white matter health and identifies several tracts that are important for cognitive performance in HD patients.

Disclosures: W.T. Richerson: None. **D.F. Wolfgram:** None. **B.D. Schmit:** None.

Poster

247. Cognitive Aging Disorders in Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 247.11/Z6

Topic: H.02. Human Cognition and Behavior

Support: NIA AG033570
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AG062251
AG061628 (O.L.)
the Canadian institutes of Health Research (MT-28414037, MOP-81112) and the Jack Bell Chair in Schizophreina (W.G.H.)
AG17917

Title: The association between adult human neurogenesis, cognition and Alzheimer's disease

Authors: *A. M. DISOUKY[1], M. K. TOBIN[1], K. MUSARACA[1], A. SHETTI[1], A. BHERI[1], W. G. HONER[2], N. KIM[3], R. J. DAWE[3], D. A. BENNETT[3], K. ARFANAKIS[3], O. LAZAROV[1];
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Abstract: The existence and extent of neurogenesis in the adult human hippocampus has been a topic of debate in recent years. Here, we provide evidence for the persistence of neurogenesis not only in aging individuals with no cognitive impairments, but also in the brains of patients with mild cognitive impairments (MCI) and Alzheimer's disease (AD). We show that neural progenitor cells, neuroblasts and immature neurons are distributed throughout the ventral-dorsal axis of the hippocampus. The number of neuroblasts and immature neurons is significantly reduced in MCI.
In addition, higher number of neuroblasts (DCX+PCNA+) is associated with better cognitive diagnosis. Furthermore, we show that the number of newly generating neurons correlates with the expression of key presynaptic proteins, previously shown to be associated with cognitive reserve. These findings imply that a higher number of neuroblasts is less likely to be associated with cognitive decline. In addition, these observations suggest that neurogenesis is compromised in an early stage of cognitive deterioration. Taken together, these results suggest that hippocampal neurogenesis persists in the brain throughout life and that enhancing neurogenesis may support cognitive function in AD.

Disclosures: A.M. Disouky: None. **M.K. Tobin:** None. **K. Musaraca:** None. **A. Shetti:** None. **A. Bheri:** None. **W.G. Honer:** None. **N. Kim:** None. **R.J. Dawe:** None. **D.A. Bennett:** None. **K. Arfanakis:** None. **O. Lazarov:** None.

Poster

247. Cognitive Aging Disorders in Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

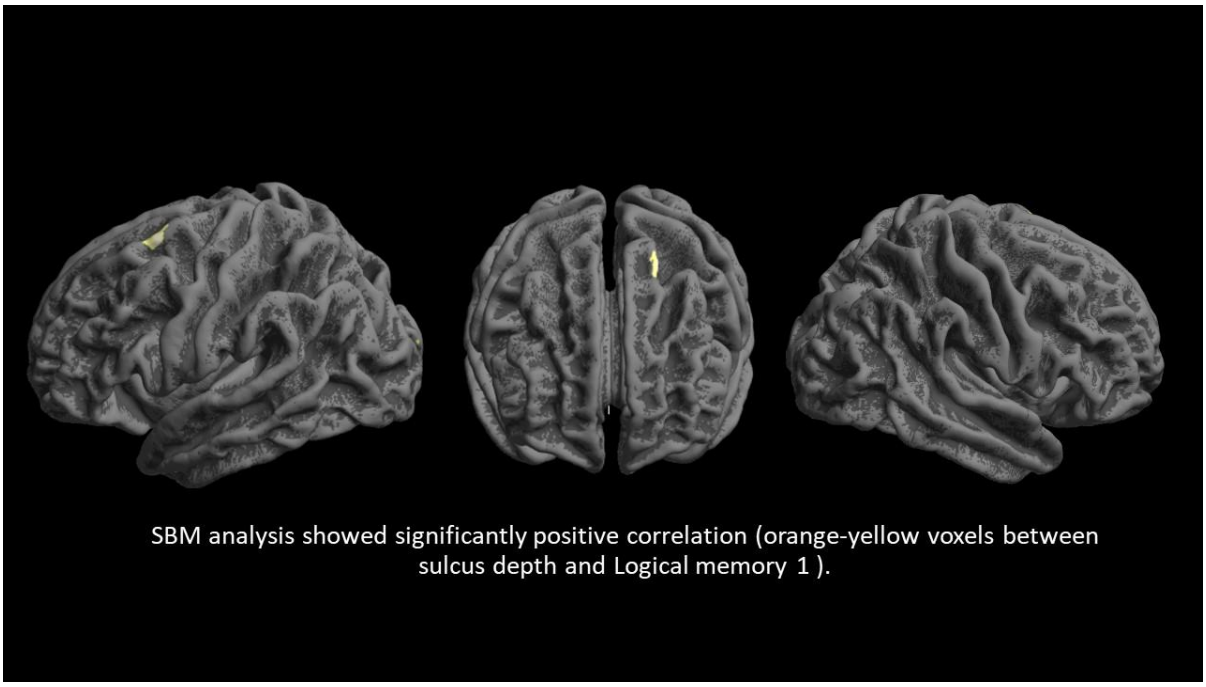
Program #/Poster #: 247.12/Z7

Topic: H.02. Human Cognition and Behavior

Title: Altered cortical thickness and curvature of the prefrontal lobe after home-based cognitive training

Authors: *A. HATTORI[1], M. KURAMOCHI[3], Y. MOTOI[2], Y. SHIMO[4], K. KAMAGATA[1], S. AOKI[1];
[1]Dept. of Radiology, [2]Dept. of Diagnosis, Prevention and Treatment of Dementia, Juntendo Univ. Sch. Med., Tokyo, Japan; [3]Radiological Sci., Tokyo Metropolitan Univ. Grad. Sch. of Human Hlth. Sci., Tokyo, Japan; [4]Dept. of Neurol., Juntendo Univ., Bunkyo-ku/Tokyo, Japan

Abstract: *Aim*: A growing body of studies indicate benefits of cognitive and physical training in patients with mild cognitive impairment (MCI), however the underlying neuronal mechanisms remain unclear. *Methods*: 24 patients with MCI were instructed to perform training exercises three times per week for 12 weeks at home accompanied with their caretakers. The training includes two-digit number calculation, reading a short original story, fill-in-the blank tasks, simple calculations and dual task training. The caretakers measured the time spent in all tasks, and they took around 40 minutes on average. MRI and 9 neuropsychological tests were undertaken before and after the training. The MRI scanning was performed on 3.0 T systems (Prisma; SIEMENS). In MRI analysis, the alteration in surface-based morphometry (SBM) was analyzed using correlation of change in score of two significantly improved neuropsychological tests. The parameters of SBM were evaluated using the computational anatomy toolbox (CAT12) for SPM. Finally, we assessed the relationships between surface indices and scores of neuropsychological tests. *Results*: 7 patients dropped out of the study and 17 patients (78.6 ±5.0 years) were analyzed. The scores of digit span (forward, backward and total score) and Logical Memory I (LM I) were significantly improved ($p = .0003$, .0136, .0002, and .0078 respectively). The cortical thicknesses of the left superior frontal gyrus (L-SFG) and frontal pole (FP) were negatively correlated with the score of digit span total points (DST) and forward (DSF). The sulcus depths of the L-SFG and right middle frontal gyrus were positively correlated with LM I and the gyrus index of the right FP showed positive correlation with DST ($p<.001$). *Discussion* : Our short-term home-based cognitive training improved executive abilities and working memory synchronized with getting the prefrontal cortex stretched, and getting the structures angulated and sculpted. Home-based cognitive training may be helpful to prevent MCI from developing into dementia, especially related to the areas of excecutive controls.



SBM analysis showed significantly positive correlation (orange-yellow voxels between sulcus depth and Logical memory 1).

Disclosures: **A. Hattori:** None. **M. Kuramochi:** None. **Y. Motoi:** None. **Y. Shimo:** None. **K. Kamagata:** None. **S. Aoki:** None.

Poster

247. Cognitive Aging Disorders in Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 247.13/Z8

Topic: H.02. Human Cognition and Behavior

Support: DST INSPIRE Fellowship (IF150288)

Title: Pre-probe EEG microstates of visual Sternberg task: A biomarker of cognitive dysfunction in primary open angle glaucoma

Authors: *R. SAMANCHI[1], S. MUTHUKRISHNAN[1], T. DADA[2], R. SIHOTA[2], S. KAUR[1], N. MEHTA[1], R. SHARMA[1];
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Abstract: Background: Glaucoma is an optic neuropathy which occurs due to the damage of retinal ganglionic cells leading to irreversible blindness. Glaucoma is considered to be a neurodegenerative disorder with cognitive deficits including working memory. Responses in a cognitive task is known to be determined by EEG microstates which is considered to be "the building blocks of cognition". Hence, we aimed to investigate the pre-probe EEG microstates in patients with glaucoma. **Materials and methods:** Verbal visual Sternberg task (VS) was administered in thirty-seven patients with primary open angle glaucoma (POAG) and 32 healthy controls (C) to assess working memory. High density EEG data was acquired with a sampling rate of 1000 Hz. Fifty milliseconds of pre-probe EEG data (1-100 Hz) was segmented from the correct trials of VS. After removing artifactual components using independent component analysis, microstate analysis was carried out using CARTOOL software. Microstate maps with least cross-validation value were identified as template maps. Number of time frames (TF), global explained variance (GEV), mean duration (MD) and time coverage (TC) of the two template maps (class A & C of Lehmann et al.,) were compared between groups using unpaired t-test ($p<0.05$). **Results:** Patients with POAG had significantly lower correct responses (C= 16±2; POAG= 13±4; p= 0.01) with higher reaction times (C= 1041±280; POAG= 1358±491; p= 0.002). For map 1 (class C), patients with POAG had significantly lower TF (C= 139±45; POAG = 110±41; p=0.02), MD (P= 25.4±13; POAG =16.6±5.7; p=0.003) and TC (C= 0.65±0.16; POAG = 0.55±0.16; p=0.04). For map 2 (class A), patients with POAG had significantly higher GEV (C= 0.059±0.04; POAG = 0.09±0.05; p=0.03) and TC (C= 0.34±0.16; POAG= 0.44±0.16; p=0.04). **Conclusion:** Shortened duration of map 1 and lengthening of map 2 representing brain activity in patients might have contributed to correct responses in VS despite lower accuracy and higher reaction times. Therefore, these microstate maps can be used as a biomarker to predict correct responses for VS task in patients with POAG.

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Poster

247. Cognitive Aging Disorders in Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 247.14/Z9

Topic: H.02. Human Cognition and Behavior

Title: Eye tracking-based cognitive assessment as a screening tool for the early detection of cognitive impairment

Authors: *A. OYAMA[1], S. TAKEDA[2], Y. ITO[4], T. NAKAJIMA[3], Y. TAKAMI[5], Y. TAKEYA[5], K. SUGIMOTO[5], K. YAMAMOTO[5], H. RAKUGI[5], R. MORISHITA[4];
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Abstract: A rapid growth in the number of people with dementia is becoming one of the biggest global issues. Recent research has shown that early diagnosis and timely intervention can prevent cognitive decline. Neuropsychological tests, such as the Mini-Mental State Examination (MMSE), are commonly used as a screening tool to detect cognitive impairment. These traditional neuropsychological tests are valid and reliable; however, they are not sufficiently simple and rapid as routine screening tools. Here, we report a newly developed eye tracking-based cognitive assessment tool to detect cognitive impairment. The gaze points of the subjects were recorded by the eye-tracking device while a series of short (178 s) task movies are displayed on the monitor, and the cognitive scores are determined from the gaze plots data. Eighty participants, including 27 cognitively healthy controls (HC), 26 patients with mild cognitive impairment (MCI), and 27 patients with dementia, were assessed by both an eye tracking-based and neuropsychological tests. A strong positive correlation was observed between the MMSE and eye tracking-based cognitive scores ($r = 0.74$, $p < 0.00001$, Spearman's rank test). The eye tracking-based cognitive scores also correlated well with other neuropsychological tests such as FAB and ADAS-cog, and they also demonstrated a good diagnostic performance in detecting MCI. Eye tracking-based cognitive assessment provides a new platform for a quantitative scoring and sensitive detection of cognitive impairment.

Disclosures: **A. Oyama:** D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); JVC KENWOOD Corporation. **S. Takeda:** D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); JVC KENWOOD Corporation. **Y. Ito:** None. **T. Nakajima:** None. **Y.**

Takami: None. **Y. Takeya:** None. **K. Sugimoto:** None. **K. Yamamoto:** None. **H. Rakugi:** None. **R. Morishita:** None.

Poster

247. Cognitive Aging Disorders in Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 247.15/Z10

Topic: H.02. Human Cognition and Behavior

Support: Grant from EU Interreg Programme
Managed by SEUPB

Title: Can current cognitive and functional assessments for Alzheimer's disease diagnosis be optimized? A data analytics approach

Authors: *N. MCCOMBE[1], X. DING[1], G. PRASAD[1], S. TODD[3], D. P. FINN[4], P. L. MCLEAN[2], **K. WONG-LIN**[1];
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Abstract: Cognitive and functional assessments (CFAs) are one of the most often used tools for dementia diagnosis in clinical care settings. However, conducting some of these assessments can take a long time with respect to the typical brief clinical consultation time. Hence the process is currently sub-optimal. In this project, we aim to improve the efficiency of conducting CFAs for AD diagnosis by applying a suite of data feature selection methods to identify a group of CFA sub-features most relevant to AD severity. We made use of CFA data from the open Alzheimer's disease NeuroImaging Initiative (ADNI) dataset. MoCA, MMSE, ADAS-Cog, FAQ, NPI, NPI-Q, Ecog, GDS, and the Neuropsychological Battery were amalgamated into one dataset along with demographic features (age, handedness, APOE4 genotype, education.) Individual samples without all these data features were dropped, leaving a sample with 380 individuals over a total of 1839 visits over a 12-year period. We used Clinical Dementia Rating Sum of Boxes (CDRSB) as an objective outcome variable, which was recoded into three classes of AD severity - Healthy Control (HC), Mild Cognitive Impairment (MCI), and Alzhiemer's Disease (AD). Non-numeric and non-ordinal features were removed, while numeric features were normalized. We used Correlation-based Feature selection (CFS), which selects an optimal subset of features, and Random Forest Importance (RFI), One Rule (OneR) and Information Gain (IG) methods, which rank features, to identify the features most relevant to CDRSB. There was high agreement among the algorithms. Of the 125 sub-features, CFS selected an optimal subset of 23, 12 of which were ranked in the top 23 by all other methods. A further 11 features were in the top 23 of at least two

methods. A Random Forest classifier based on the top 23 sub-features selected by RFI achieved 84.6% accuracy over three classes, suggesting potential for an optimised CFA based on these sub-features. Accuracy did not decline significantly with a shorter test; a Random Forest classifier based on the 12 features which were selected by all 4 methods achieved 81% accuracy. Additionally, 80% prediction accuracy in predicting progression from MCI to AD was achieved using the top 23 features selected by RFI and a Random Forest Classifier. Although predicting progression from HC to MCI based on CFA sub-features was not possible, 4 sub-features were identified as strongly associated with progression probability. Overall, our approach is promising towards developing a more efficient CFA to be integrated into a clinical decision support system. Future work will incorporate biomarkers into the analyses for diagnosis and prognosis prediction.

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Poster

247. Cognitive Aging Disorders in Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 247.16/Z11

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01NSS4040

Title: Cognitive dysfunction and sex differences in Parkinson's disease

Authors: *T. H. REEKES[1], C. I. HIGGINSON[4], C. R. LEDBETTER[2], N. SATHIVADIVEL[3], R. M. ZWEIG[3], E. A. DISBROW[3];
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Abstract: BACKGROUND:
Parkinson’s disease (PD) is a progressive neurodegenerative disorder that is 1.5 times more common in men than women with no clear etiological factor. PD is predominantly a motor disorder; however, cognitive function is also disrupted.
OBJECTIVE:
Because of inherent gender bias we tested the hypothesis that there are sex differences in cognitive dysfunction in PD.
METHODS:
We examined 88 participants (38 female) with PD and 59 controls (27 female), obtaining demographic variables, UPDRS and various cognitive domains: attention and working memory (WAIS-III), visuospatial and executive function (D’KEFS), and processing speed (SDMT), as

well as instrumental activities of daily living (TIADL). All evaluations were done at best ON in early stage PD.

RESULTS:

Men showed consistently poorer performance on tasks of visuospatial function (D'KEFS Trail Making Test (scanning; $F(1,86) = 5.331$, $p = 0.023$), switching (D'KEFS 4-5; $F(1,86) = 5.860$, $p = 0.018$), inhibition (D'KEFS Color Word Interference; $F(1,86) = 6.280$, $p = 0.014$), and verbal fluency (D'KEFS category naming, category switching and total switching accuracy; $F(1,86) = 14.577$, $p < 0.001$; $F(1,86) = 14.089$, $p < 0.001$; $F(1,86) = 7.952$, $p = 0.006$). Finally, men had significantly slower speed of processing ($F(1,86) = 7.481$, $p = 0.008$), which after regression analysis was the only factor associated with significant impairments in the Timed Instrumental Activities of Daily Living (TIADL; ß = -0.672, $t(48) = -6.291$, $p < 0.0001$).

CONCLUSIONS:

Our data indicate that men with PD have significantly greater cognitive impairments compared to women despite no differences in disease severity age, years of education, premorbid IQ, general cognitive function, depression, or daytime sleepiness. Our data are consistent with the finding that men present more frequently with postural instability and gait disturbance (PIGD) compared to women. PIGD is associated with an executive dysfunction phenotype.

Disclosures: T.H. Reekes: None. **C.I. Higginson:** None. **C.R. Ledbetter:** None. **N. Sathivadivel:** None. **R.M. Zweig:** None. **E.A. Disbrow:** None.

Poster

247. Cognitive Aging Disorders in Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 247.17/Z12

Topic: H.02. Human Cognition and Behavior

Support: LSUHSC Shreveport Institutional Grant in Aid

Title: Alzheimer's disease research participation in underserved minorities

Authors: N. GLASSY[1], C. ARNOLD[2], T. DAVIS[2], *E. DISBROW[1];
[1]Neurol., [2]Med., LSUHSC, Shreveport, LA

Abstract: There is a 2-fold greater prevalence of Alzheimer's disease (AD) in the African American (AA) compared to Caucasian (C) population. Yet minority elderly patients are less likely to enroll in research trials, a situation which is exacerbated when low healthcare literacy or below average socioeconomic status are also present. In the case of eldercare and age-related neurodegenerative disease, the knowledge and beliefs about research of the home caregiver take on particular importance. Thus our goal was to examine caregiver knowledge, sources of information and willingness to participate in AD research in rural areas in the Deep South.

We used a mixed methods design integrating qualitative (focus group) and quantitative (survey) data gathered in Council on Aging sites, Health Fairs, AA sororities and churches in Northwest Louisiana.
Eight focus groups (59 adults; 92% female, 78% African American) revealed that knowledge about AD came mostly from observing their loved one. Quantitative findings from a survey given to 117 caregivers (83% female, 72% African American, 30% limited heath literacy, 27% low income) found sources of AD information were their loved one's doctor (54%), friends/relatives (32%), books (31%) and health fairs (30%). AA participants (35%) were more likely than C participants (16%) to get information from TV, with a similar trend for the internet (AA 32%, C 44%). Most participants reported they had a computer, internet access, or a smart phone, but less than half reported being comfortable using it. In the focus groups caregivers reported not being asked to participate in research. Concerns about research participation were privacy (30% concerned), participant distress (27%), convenience (11%) expense (13%) and distrust of doctors (3%). Helping others (33%), personal benefit (17%) and payment (10%) were positive outcomes. There were no differences across racial groups.
Surprisingly, there were no racial differences in concerns about research participation, and distrust of doctors was indicated by only 3% of participants. Our findings indicate that a tailored strategy could improve research recruitment of underserved minorities for Alzheimer's disease research.

Disclosures: **N. Glassy:** None. **C. Arnold:** None. **T. Davis:** None. **E. Disbrow:** None.

Poster

247. Cognitive Aging Disorders in Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 247.18/Z13

Topic: H.02. Human Cognition and Behavior

Support: NIH NRSA fellowship F32 AG058479-02

Title: Measuring age-related changes in locus coeruleus intensity and its relationship to cognitive aging

Authors: *E. RILEY[1], S. STEINBERG[1], L. CHEN[1], K. M. SWALLOW[2], E. D. DE ROSA[1], A. K. ANDERSON[1];
[1]Dept. of Human Develop., [2]Psychology, Cornell Univ., Ithaca, NY

Abstract: Despite decades of research into the causes of neurodegenerative diseases such as Alzheimer's disease, these conditions still have devastating consequences. New approaches are desperately needed. The locus coeruleus (LC), a brainstem nucleus that produces the neurochemical norepinephrine (NE), and regules arousal and attention, is one of the earliest sites

of damage in AD. The role of the LC in neurodegenerative conditions remains largely underexplored. Despite the possibility that LC/NE dysfunction may serve as an early warning sign of dementia, due to being one of the first sites of neurodegeneration, few links between LC function and cognitive dysfunction have been established. There are several mechanisms by which LC integrity and increased NE signaling may protect the brain from neurodegeneration. NE has also been shown to protect LC and cortical neurons from beta-amyloid toxicity. Neuromelanin, a pigment produced in LC neurons, may protect against oxidative damage. Moreover, the LC/NE system is proposed to be a primary mediator of cognitive reserve. Given these important associations between the functions of the LC and neurodegeneration, our study was designed to establish a link between multiple measures of LC function and cognitive health in younger (n = 14) and older adults (n = 16). We assessed basic cognitive ability using the NIH Toolbox and also the Trail-Making Test B, screened for neurodegenerative disease using the Montreal Cognitive Assessment, and estimated crystalized cognitive ability using the National Adult Reading Test. The size and intensity of the LC was measured using a neuromelanin-sensitive MRI scan. BOLD activity in the LC during a attention task was measured using multi-echo fMRI. As pupil diameter is dependent on LC neuronal activity in the absence of changes in luminance, infrared pupillometry was carried out concurrently to measure task-evoked pupillary responses. This afforded an examination of a non-invasive measures LC integrity across age groups. Preliminary data shows a significant relationship ($p = 0.018$) between cognitive function and locus coeruleus intensity in older but not younger adults (Pearson correlation between NIH Toolbox fluid composite cognition score, $r = -0.62$ for older adults, $r = -0.07$ for younger adults). This results indicates that greater neuromelanin intensity was associated with poorer cognitive performance in older adults in our study. A negative correlation could be a consequence of neuromelanin's biochemical role in sequestering environmental toxins. This finding will be contextualized with full analysis of our behavioral, neuropsychological, pupillary and fMRI data.

Disclosures: E. Riley: None. **S. Steinberg:** None. **L. Chen:** None. **K.M. Swallow:** None. **E.D. De Rosa:** None. **A.K. Anderson:** None.

Poster

247. Cognitive Aging Disorders in Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 247.19/Z14

Topic: H.02. Human Cognition and Behavior

Title: Mild and severe cognitive impairment by MoCA test in older adults of a Mexico population is related with their education level

Authors: A. CÁRDENAS-PÉREZ[1], E. DOMÍNGUEZ-GÓMEZ[1], R. SALAS-CORONADO[2], N. SANTOS-SÁNCHEZ[2], B. HERNÁNDEZ-CARLOS[2], M. MERAZ-RÍOS[3], ***K. LIRA-DE**

LEON[1];
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Abstract: Diseases related to older people have become a serious public health problem worldwide. Aging is associated with a faster rate of cognitive decline either type mild or severe cognitive impairment and is considered a risk factor for developing Alzheimer disease (AD). Early diagnosis is essential to stop the progression of the cognitive decline, lengthening the patient's life and improving their quality of life. The neuropsychological evaluation can be measured by the use of brief, standardized and simple tests such as the MoCA test, which is a screening instrument with good results. The use of these tests has shown that there are several factors that affect the development or not of AD. In the present study, the relationship between educational level and the results of an assessment of cognitive impairment is evaluated using the MoCA test in a Mexico population. The MoCA test was applied to 64 patients from 58 to 93 years of age, obtaining a significant tendency towards deterioration, depending not on sex or age, but rather on the years of schooling. The patients considered illiterate (cannot read or write) tend to present a greater deterioration (severe cognitive impairment). With the previous results we can demonstrate, that the stimulation of the central nervous system is fundamental, to diminish the risk to develop cognitive impairment.

Disclosures: A. Cárdenas-Pérez: None. **E. Domínguez-Gómez:** None. **R. Salas-Coronado:** None. **N. Santos-Sánchez:** None. **B. Hernández-Carlos:** None. **M. Meraz-Ríos:** None. **K. Lira-De Leon:** None.

Poster

247. Cognitive Aging Disorders in Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 247.20/Z15

Topic: H.02. Human Cognition and Behavior

Title: ERP changes linked to spatial attentional losses in aging and early Alzheimer’s

Authors: *C. T. LOCKWOOD[1], C. J. DUFFY[2];
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Abstract: OBJECTIVE: The early detection of Alzheimer’s disease requires our distinguishing it from cognitive aging. We study visuospatial processing in a spatial attention task by recording behavioral and ERP responses in young (YN) and older (ON) normal adults, and early-stage AD (EAD) patients. Here we test whether spatial inattention might support that distinction. METHODS: We engaged young (YN) and older (ON) normals, and early Alzheimer’s disease

(EAD) patients in an attentionally cued, self-movement heading discrimination task while we recorded push-button RTs and ERPs. RESULTS: All groups learned and performed the cued heading discrimination task. YNs and ONs showed attentional cueing effects, whereas EADs did not ($p < .001$). Likewise, YNs and ONs showed shifting of ERP lateralization with attentional cues, whereas EADs did not ($p < .001$). These changes were accompanied by changes in signal properties: ON's show increased occipito-parietal sensory response power with decreased fronto-parietal cognitive response synchrony. EADs show losses of both power and synchrony. CONCLUSIONS: Our findings suggest that spatial inattention in EAD that may reflect losses in cortico-cortical signal transmission and contribute to heading direction processing impairments that undermine navigational capacity and driving safety.

Disclosures: C.T. Lockwood: None. **C.J. Duffy:** None.

Poster

247. Cognitive Aging Disorders in Humans

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Program #/Poster #: 247.21/Z16

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant 1K25AG050759-01A1

Title: Tract specific white matter reductions in amnestic mild cognitive impairment

Authors: *E. GOZDAS, H. FINGERHUT, L. CHROMIK, H. HOSSEINI;
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Abstract: Introduction: Mild cognitive impairment (MCI) represents a translational period of cognitive function between the changes seen in normal aging and those fulfilling the features for early Alzheimer's disease[1]. MCI patients identified with progressive memory deficits either alone or in conjunction with impairments in other cognitive domains are defined as amnestic MCI (aMCI) that are likely in the prodromal stage of Alzheimer's disease. A number of recent neuroimaging studies have shown atypical white matter properties in various components of the memory circuitry in aMCI. However, there is a gap in fine-grained examination of white matter tracts to accurately characterize how and where white matter microstructure varies in aMCI patients. The objective of this study is to examine changes in tract-specific white matter microstructures along two tracts thought to carry signals critical for memory functions: the left and right cingulum cingulate in aMCI patients.**Methods:**Twenty-one patients with a diagnosis of aMCI (age range 65-85 years) and 24 age- and education-matched healthy controls (age range 65-85 years) were recruited for the study. The aMCI patients were diagnosed based on a comprehensive evaluation including neuropsychological testing and clinical history.Fiber tracts were identified for each subject using the Automated Fiber Quantification (AFQ) software

package, after initial generation of a whole-brain connectome using deterministic tractography in MRtrix. Fiber tracking was carried out on aligned, distortion and motion corrected multi-shell diffusion tensor imaging (DTI) data. Selected white matter tracts were mapped into 30 equidistance nodes and then diffusion properties (Fractional Anisotropy (FA) and Mean Diffusivity (MD)) were mapped onto each tract. **Results:**Diffusion characteristics of the cingulum cingulate tract were affected in aMCI compared with controls but mainly in the middle part of the tract. Particularly, we found significantly decreased FA and increased MD values in nodes 6 to 25 along the left and right cingulum cingulate in aMCI patients compared to healthy controls ($P<0.05$, FDR-corrected). **Conclusions:**The change in white matter tissue properties in fine-grain level is highly relevant to memory decline with ageing that can be used as a model of the biological principles underlying later cognitive decline that are critical for the understanding the process of the microstructural deterioration of the brain's connective pathways for neurodegenerative diagnosis in older adults.**References:1**-Petersen, Ronald C. "Mild cognitive impairment." New England Journal of Medicine 364.23 (2011): 2227-2234

Disclosures: E. Gozdas: None. **H. Fingerhut:** None. **L. Chromik:** None. **H. Hosseini:** None.

Poster

247. Cognitive Aging Disorders in Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 247.22/Z17

Topic: H.02. Human Cognition and Behavior

Title: A smRNA-seq study using post-mortem brain tissue samples in patients with Alzheimer's disease compared to cognitively normal control subjects

Authors: D. CAI, ***Q. S. LI**;
Janssen R&D LLC, Pennington, NJ

Abstract: Alzheimer's disease, the leading form of dementia, is associated with abnormal tau and β-amyloid accumulation in the brain, leading to tangle and plaque formation. However, the underlying cause of the disease remains unknown. In this study, we conducted a smRNA-seq study to identify small RNAs associated with Alzheimer's disease in post-mortem brain samples from the inferior frontal gyrus (IFG), middle temporal gyrus (MTG), and superior temporal gyrus (STG). We observed several miRNAs that have previously been implicated in Alzheimer's disease, including miR-212 and miR-132 downregulation in Alzheimer samples across all three brain regions. Downregulation of miR-339-5p and miR-144-3p and upregulation of miR-128-1-5p in the STG, as well as downregulation of miR-431 and miR-137 and upregulation of miR-206 and miR-221 in the MTG were also detected. Additionally, we observed a few miRNAs upregulated in the brain that have previously been found to be upregulated in the blood. These include miR-502-3p and miR-30a-3p in the STG and miR-206 in the MTG. We have also

observed differential expression across dozens of other miRNAs in the STG and MTG that have not previously been described. These miRNAs can be further validated experimentally and may be cross-validated in blood samples to determine if they can serve as diagnostic markers for Alzheimer's disease. Altogether, we identified differential expression in several miRNAs previously associated with Alzheimer's disease in our brain samples and nominate dozens of under-studied miRNAs for further examination in their ties to Alzheimer's disease progression and their uses as diagnostic biomarkers.

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Poster

247. Cognitive Aging Disorders in Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 247.23/Z18

Topic: H.02. Human Cognition and Behavior

Support: NIA Grant P01-AG026276
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NIA Grant T32-AG000030-32
NSF Grant IGERT-0548890

Title: Evaluating bold variability as a function of age and as a potential biomarker of Alzheimer's disease

Authors: *P. R. MILLAR, D. A. BALOTA, S. E. PETERSEN, B. M. ANCES, B. A. GORDON, S. E. SCHINDLER, A. M. FAGAN, T. L. S. BENZINGER, J. C. MORRIS; Washington Univ. In St. Louis, St. Louis, MO

Abstract: A growing body of research in cognitive neuroscience of human aging reports that variability in blood oxygen-level dependent (BOLD) signal in functional MRI is negatively related with chronological age and positively related with behavioral task performance. These findings have been interpreted to suggest that BOLD variability may reflect an age-dependent, functionally-relevant signal. However, further work is needed to rule out potential methodological confounds, such as individual differences in head motion. BOLD variability may also be related to preclinical Alzheimer disease (AD) pathology, and hence, afford unique clinical utility. We pursued these aims using a support vector regression machine learning approach in a large (N = 422) sample of human resting-state fMRI scans from cognitively normal individuals ranging in age from 40 to 80 years old. Confirmatory analyses attempted to replicate the previous negative relationship between BOLD variability and age after applying

stricter controls for head motion, including global signal regression and framewise motion censoring. We replicated the negative relationship with age, while substantially reducing relationships with head motion, suggesting that this effect is robust above and beyond individual differences in head motion. Further analyses of anatomical specificity indicate that the age relationship is broadly distributed throughout a wide variety of functional networks, suggesting that a global mechanism might best account for this effect. Our exploration of BOLD variability and cerebrospinal fluid (CSF) biomarkers of preclinical AD yielded small relationships between BOLD variability and CSF amyloid, but no relationships with CSF tau or phosphorylated tau. However, BOLD variability was more strongly related to hippocampal volume. Our results support the general finding that age is associated with reduced BOLD variability. Regarding preclinical AD, our results suggest that BOLD variability may be sensitive to later neurodegenerative processes (as reflected by hippocampal volume), but only weakly related to earlier accumulation of amyloid and tau. Future studies will be helpful for isolating the mechanism behind these relationships.

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Poster

247. Cognitive Aging Disorders in Humans

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Taylor Family Institute for Innovative Psychiatric Research, Washington University
Center for Brain Research in Mood Disorders,Washington University
Saint Louis University

Title: Effects of computerized cognitive training on cognition and resting state brain networks in older adults

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Abstract: Age-related cognitive decline affects a broad range of fluid cognition domains including recall, executive function, and information processing speed. It is a potentially treatable phenomenon, with opportunities for intervention to slow or reverse the decline in late life. One area of opportunity for intervention is cognitive training. However, a common critique of the existing cognitive training literature is the often-observed low effect size. The objective of the present study was to determine whether effects of an intensive computerized cognitive training intervention could be amplified with addition of a putatively pro-cognitive medication. The FDA-approved antidepressant vortioxetine has been reported to improve cognition across several domains, above and beyond any benefits of alleviating symptoms of depression. In this study, older adults (age 65+) who reported age-related cognitive difficulties were randomly assigned to receive 6 months of intensive, adaptive computerized training on a broad range of cognitive domains along with either vortioxetine or a placebo. A subset of the sample completed structural and functional MRI at the start and end of the training period. The primary objectives of this analysis were to measure cognitive changes and brain changes as a function of the training period and drug intervention. The study outcome measures were the NIH Toolbox Cognitive Battery fluid cognition composite score and resting state fMRI network changes. There was overall improvement in cognition from baseline to 6 months, as measured by NIH Toolbox scores. At baseline, stronger resting state coherence within the Default Mode Network (DMN) and within an attention-related network corresponded with better cognition. We also observed pre- versus post-training changes in resting state networks that differed as a function of drug intervention group. In particular, drug and placebo groups differed in pre-post DMN coherence and relationship of DMN to control networks. In conclusion, combining cognitive training with vortioxetine for 6 months produced greater improvements in cognitive performance as well as changes in resting-state brain networks. For older adults experiencing age-related cognitive decline, pro-cognitive medication added to cognitive training may have additive or synergistic beneficial effects.

Disclosures: J.D. Waring: None. **S.E. Williams:** None. **A. Pogarcic:** None. **E.J. Lenze:** 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.; Takeda, Lundbeck.

Poster

247. Cognitive Aging Disorders in Humans

Location: Hall A

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant P50AG005681
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NIH Grant P01AG026276
NIH Grant UF1AG032438

Title: Impact of CSF Ab1-42 on functional brain network dynamics and cognitive intraindividual variability in older adults

Authors: *K. L. MEEKER[1], B. M. ANCES[2], B. A. GORDON[2], A. M. FAGAN[2], D. A. BALOTA[2], T. L. BENZINGER[2], J. C. MORRIS[2], J. D. WARING[1];
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Abstract: In cognitively normal older adults, functional brain networks, such as the dorsal attention network (DAN), show greater neural recruitment in response to attention demanding tasks than at rest, whereas the default mode network (DMN) shows the opposite pattern. The inverse relationship, or anticorrelation, between the two networks likely reflects efficient competition for cognitive resources. As Alzheimer Disease (AD) related pathology accumulates, prior to cognitive dysfunction, functional connectivity within and between networks breaks down. This breakdown also increases within-person inconsistency in performance on cognitive tasks (cognitive intraindividual variability, IIV). We hypothesized that (1) functional connectivity between the DAN and DMN will be dysregulated with increasing disease progression; (2) IIV across neuropsychological domains (calculated as the individual standard deviation) will be greater in individuals with mild AD (Clinical Dementia Rating Scale; CDR=0.5-1) than in cognitively normal older adults (CDR=0); and (3) the IIV-anticorrelation relationship will weaken with increasing disease progression. Neuroimaging, cerebrospinal fluid (CSF), and cognitive measures from the Alzheimer's Disease Neuroimaging Initiative (ADNI) and Washington University's Knight Alzheimer's Disease Research Center (ADRC) were used to assess within and between network functional connectivity and IIV in cognitively normal older adults and those with mild AD. Results showed cognitive IIV was greater in mild AD than in cognitively normal older adults. The relationship between cognitive IIV and the DMN-DAN anticorrelation was weaker for individuals with mild AD than cognitively normal individuals. Exploratory mediation analyses indicated that CSF Ab1-42, but not tau (a marker of neuronal injury), mediates the relationship between cognitive IIV and DMN-DAN anticorrelation. Collectively, our results suggest that in mild AD overall cognitive functioning becomes less consistent and decreases in cognitive consistency are associated with the accumulation of CSF marker of amyloid (Ab1-42) and breakdowns in functional network anticorrelations. This study provides novel insight into how amyloid burden and alterations in the dynamics of functional networks give rise to behavioral changes in mild AD.

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Poster

247. Cognitive Aging Disorders in Humans

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Topic: H.02. Human Cognition and Behavior

Support: NIA Grant R21-AG-045460
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Title: Aging and working memory training: Testing the compensation related utilization of neural circuits hypothesis (CRUNCH)

Authors: *A. D. IORDAN[1], K. A. COOKE[1], K. D. MOORED[2], B. KATZ[3], M. BUSCHKUEHL[4], S. M. JAEGGI[5], T. A. POLK[1], S. J. PELTIER[1], J. JONIDES[1], P. A. REUTER-LORENZ[1];
[1]Univ. of Michigan, Ann Arbor, MI; [2]Johns Hopkins Univ., Baltimore, VA; [3]Virginia Tech., Blacksburg, VA; [4]MIND Res. Inst., Irvine, CA; [5]Univ. of California, Irvine, CA

Abstract: CRUNCH posits that additional neural resources are recruited with increasing task demand regardless of age. However, older adults over recruit at lower levels of demand compared to younger adults, reaching capacity sooner with activation decline at higher loads (quadratic CRUNCH curve relating memory load and activation). We hypothesized that working memory (WM) training would bolster neural efficiency, lowering activation so that the activation peak shifts towards higher WM loads after training. We recruited 23 older and 23 young participants for an adaptive verbal WM training study. The study protocol comprised 3 functional MRI (fMRI) sessions, with the first 2 sessions (s1, s2) 2 weeks apart, preceding training, and s3 conducted immediately after a 10-day training intervention. This design permitted the dissociation of task-exposure (s1 vs. s2) and training (s2 vs. s3) effects. During each fMRI session, participants performed a delayed match-to-sample verbal WM task with span and supra-span loads. Behaviorally, performance improved with training for both groups. Regarding brain activity, 3 sets of analyses examined: (1) task-exposure and training effects between groups, using a meta-analytical WM map, (2) training effects within each group, using age-specific maps identified at s1, and (3) region-specific training effects in two load-sensitive prefrontal cortex regions identified at s1. These analyses revealed minimal task exposure effects and convergent results for training-related plasticity. First, both groups showed increased recruitment of canonical WM regions at higher loads due to training, with a rightward shift of the CRUNCH curve specific to older adults. Second, group-specific analyses replicated these effects in task-positive regions, whereas task-negative regions showed less deactivation with training, particularly for lower loads, that was specific to younger adults. Third, in right dorsolateral prefrontal cortex older adults showed a trend toward reduced recruitment, consistent with less

compensation post-training, whereas in left inferior frontal gyrus younger adults only showed greater recruitment for higher loads, suggesting greater engagement of rehearsal circuitry with training. Thus, consistent with CRUNCH, our results show that pre-training older adults over-recruit WM regions and reach peak activation at lower loads than younger adults. As predicted, however, WM training shifts this activity ceiling to higher WM loads in both age groups. This outcome suggests that training increases the dynamic range of activation in the WM circuitry, enabling greater responsiveness at higher loads, regardless of age.

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Poster

247. Cognitive Aging Disorders in Humans

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Title: Functional connectivity in autosomal dominant Alzheimer's disease (ADAD) versus late onset Alzheimer's disease (LOAD)

Authors: *J. F. STRAIN[1], A. TANENBAUM[2], A. ASCHENBRENNER[2], B. GORDON[2], J. HASSENSTAB[2], J. MORRIS[2], T. BENZINGER[2], R. BATEMAN[2], B. ANCES[2];
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Abstract: Autosomal Dominant Alzheimer Disease (ADAD) differs from late onset Alzheimer Disease (LOAD) both in its genetic determinants and age of onset. Resting state functional connectivity (FC) abnormalities have been described in both ADAD and LOAD. However, there remains uncertainty regarding the extent to which FC changes in these two forms of AD differ from the effects of normal aging. Moreover, differences in the FC manifestations and the cognitive deficits for ADAD vs. LOAD are incompletely understood. Here, we examined these questions in participants with ADAD and LOAD as well as age-matched controls. To distinguish

between the effects of healthy aging, ADAD, and LOAD, we represented FC in a space of reduced dimensionality based on principal component analysis (PCA) of FC matrices derived from 246 regions of interest. Cognitive severity was quantified from a cognitive composite z-score calculated from episodic memory, processing speed and executive function. The PCA analysis showed that the spatial topography and magnitude of effects in AD differ from those of healthy aging. Differences between ADAD vs. LOAD, controlling for age, depended on CDR stage. Specifically, the magnitude, but not spatial topography, of FC changes was greater for ADAD CDR 0.5 (very mild cognitive impairment) but was not evident at CDR 1 (moderate to severe cognitive impairment). Worse FC significantly correlated with poorer performance only for mutation positive ADAD individuals ($p=0.0016$) but not LOAD ($p=0.06$). This association for ADAD was only observed in symptomatic individuals (CDR0.5) when split by CDR status ($p<0.001$). These results demonstrate marked differences between AD vs. healthy aging. Additionally, the FC manifestations of ADAD and LOAD appear to be comparable at later stages of the disease. The FC manifestations were spatially similar but greater for ADAD compared to LOAD and cognitive decline only associated with FC in our ADAD cohort. ADAD is a more homogeneous cohort compared to LOAD due to an earlier age of onset and fewer age-related comorbidities. This implies the cognitive deficits present in that population are largely a factor of AD pathology and not compounded by secondary underlying pathologies. Together, this supports that FC is impacted by similar mechanisms across multiple networks in the brain for either ADAD or LOAD and this pattern of FC reflects AD-related cognitive decline.

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Poster

247. Cognitive Aging Disorders in Humans

Location: Hall A

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Topic: H.02. Human Cognition and Behavior

Support: P50 AG005142
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R01 AG054073
R01 AG058537

Title: Correlation between hippocampal cornu ammonis 1 thickness and default mode network connectivity at rest in non-demented older adults

Authors: *J. ZHOU[1], M. A. TUBI[1], D. KOTHAPALLI[1], N. HAZRA[1], S. I. THOMOPOULOS[1], M. D. SWEENEY[2], X. HUI[3], L. S. SCHNEIDER[3,4], E. B. JOE[3], J. M. RINGMAN[3], H. N. YASSINE[5], M. G. HARRINGTON[6], B. V. ZLOKOVIC[2,7], A. W. TOGA[3,8], H. C. CHUI[3], M. N. BRASKIE[1,3];
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Abstract: Introduction: The default mode network (DMN) has been related to episodic memory, and is affected in subjects at increased risk for Alzheimer's disease (AD) - either genetically, or because they have mild cognitive impairment (MCI). AD risk is associated with lower DMN functional connectivity with the medial temporal lobe (MTL), while MCI has been associated with both increased and decreased connectivity between the DMN and frontal cortex. We previously found that lower volumes of the subiculum (SUB) and cornu ammonis 1 (CA1) were associated with abnormal levels of β-amyloid$_{42}$ in the cerebrospinal fluid. These regions also are thinner in older adults are at increased genetic risk for AD because they carry an apolipoprotein E ε4 (*APOE4*) allele. In the current study, we evaluated the relationship between DMN connectivity and cortical thickness of SUB and CA1 among cognitively normal older adults. We hypothesized that variability in DMN connectivity with frontal cortex and the MTL would be associated with SUB and CA1 thickness. Gaining a better understanding of the mechanisms underlying these early connectivity differences may aid in designing and monitoring early interventions.
Methods: We obtained T2-weighted high resolution hippocampal (HHR) and resting-state functional MRI (rsfMRI) scans from 27 non-demented subjects recruited by the USC ADRC (age = 66 ± 6.7 years, 18 females, Global CDR Score = 0). We used the Automatic Segmentation of Hippocampal Subfields (ASHS) software to segment the HHR scans into subregions, and calculated mean cortical thickness in the bilateral CA1 and SUB. We used a seed-based (posterior cingulate cortex/precuneus) fMRI approach to identify the DMN. We evaluated the relationships between voxelwise activity in the DMN and mean bilateral SUB and CA1 thickness, covarying for age and sex. We used cluster thresholding to correct for voxelwise multiple comparisons ($Z>3.1$; $p<0.025$ corrected) and Bonferroni correction to correct for two subregion comparisons.
Results: Thinner mean bilateral CA1 was associated with greater connectivity between the DMN and the left superior frontal gyrus (Z-max=3.91, $p=7.0\text{x}10^{-4}$). *APOE4* carrier status was not

associated with fMRI activity in this region (p=0.86). SUB thickness and DMN activity were not significantly related.
Conclusions: Our findings of hyperconnectivity in those with thinner CA1 are consistent with prior findings of DMN hyperconnectivity in MCI patients and in older adults who are positive for amyloid but not tau. This may represent neuronal compensation early in the disease process, which warrants testing with longitudinal follow-up.

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Poster

247. Cognitive Aging Disorders in Humans

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 247.29/Z24

Topic: H.02. Human Cognition and Behavior

Title: Intra-individual variability of event-related potential amplitude suggests decreased neural efficiency in cognitively intact human carriers of the apolipoprotein-E ε4 allele

Authors: *E. R. PAITEL, S. A. EVANS, K. A. NIELSON;
Marquette Univ., Milwaukee, WI

Abstract: Objective and Rationale:
Limited event-related potential (ERP) research has employed single-trial variability to index subtle differences in neural processing in the context of healthy aging and Alzheimer's disease (AD). Increased amplitude and latency intra-individual variability (IIV) have been shown in older vs. young adults and in AD vs. healthy elders, suggesting decreased efficiency of neural firing. Moreover, neurological changes associated with AD begin decades before symptom onset. Despite its importance, no research to date has employed these methods with healthy, cognitively intact groups with risk for pathological aging. Whereas traditional approaches to ERP data analysis average across dozens of trials, thus eliminating trial-to-trial variations in firing, IIV capitalizes upon variance metrics, making it advantageous for detecting subtle group differences.
Methods:
Healthy, cognitively intact older adults (n=44) were distinguished by genetic risk for AD via the Apolipoprotein-E (APOE) ε4 allele (21 ε4+, 23 ε4-). ERPs were assessed during a Stop-signal inhibitory control task at midline electrodes (Fz, FCz, Cz, Pz). N200 (novel stimulus detection, attention) and P300 (executive control, conflict monitoring) components were converted to amplitude variability using *SD* of single-trial peaks.
Results:

Hierarchical regression models with age and sex in Step 1 and APOE ε4 in Step 2 revealed APOE ε4 significantly predicted greater N200 (Fz: R^2=.17; p=.05; R^2_{change}=.11) and P300 (Fz: R^2=.21; p=.02; R^2_{change}=.16; FCz: R^2=.20; p=.03; R^2_{change}=.17) amplitude variability in fronto-central electrodes in healthy, cognitively intact elders.

Conclusions:

ERP amplitude variability indicative of frontal lobe dysfunction is evident even in *healthy, cognitively intact* APOE ε4+ elders during inhibitory control, which may reflect declining efficiency of underlying inhibitory control networks. Importantly, these variability metrics may be crucial to detecting *subtle,* early differences in neural processing that may indicate risk for future cognitive decline, thus allowing for early implementation of preventative measures. Future ERP research should focus both on healthy groups with risk for future cognitive decline and employment of these sophisticated variability metrics.

Disclosures: E.R. Paitel: None. **S.A. Evans:** None. **K.A. Nielson:** None.

Poster

248. Human: Timing and Temporal Processing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 248.01/Z25

Topic: H.02. Human Cognition and Behavior

Title: Characterization of the power spectrum of the EEG in basal condition, simple learning processes and their correlation with attention and memory

Authors: *Y. M. SERRATO[1], *H. BRUST[2];
[1]Neurosciences, Univ. Nacional Autonoma De Mexico, México, City, Mexico; [2]Inst. Nacional de Rehabilitación, México, City, Mexico

Abstract: Cognition results from the synchronic interaction of bioelectric activity of the glio-neuronal assemblies that construct the brain circuits. However, to identify these circuits it is essential to describe absolute power (AP) profiles of the spectrum with its topography in *default* mode *network* (DMN) and its modifications in simple learning processes. The purpose of the present study is to simultaneously analyze four frequencies of the EEG (δ, θ, α and β) in DMN, habituation to repeated photostimulation (RPh) and visual-motor association (asso-vm) describing possible differences between genders and correlating the AP of the DMN with performance in neuropsychological evaluations. A total of 40 undergraduate students (20 ± 2 years) were divided into a group of women (GW) and a group of men (GM). [IM1] The EEG was recorded in DMN and before (pre-RPh) and during RPh of 20 series from 2 s to 5 Hz (condition of habituation). A similar RPh was performed with the indication that upon sensing it, a switch (aso-vm condition) had to be pressed with the dominant hand. Analysis of the qEEG revealed differences between genders in the power spectrum: GM showed higher AP of δ with

respect to GW, whereas in the other bands (θ, α, and β) the opposite phenomenon occurred. In habituation, synchronization activity was predominant (understood as greater inhibitory activity), whereas during asso-vm greater desynchronization was observed (greater activation). Finally, a correlation of the AP of θ in DMN with the total memory score was found. These duly identified data could be established as quantitative electroencephalography biomarkers for the support of neurological diagnoses and follow-up of cognitive neuro-rehabilitation interventions. Keywords: Glioneuronal assemblies; absolute power; topographic profiles; simple learning processes; habituation; association; correlation between electrical activity and cognitive processes

Disclosures: Y.M. Serrato: None. **H. Brust:** None.

Poster

248. Human: Timing and Temporal Processing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 248.02/Z26

Topic: H.02. Human Cognition and Behavior

Support: KAKENHI-PLANNED-18H05523

Title: Internal clock in Parkinsonism - Is it slow or fast and does it correlate with the clinical stage?

Authors: *Y. TERAO[1], T. MIYAZAKI[2], M. HONMA[3], S.-I. TOKUSHIGE[2], S. INOMATA-TERADA[1], A. UCHIBORI[2], Y. ICHIKAWA[2], A. CHIBA[2], Y. UGAWA[4], M. SUZUKI[5]; [1]Dept. of Med. Physiol., [2]Dept. of Neurol., Kyorin Univ., Tokyo, Japan; [3]Dept. of Physiol., Showa Univ., Tokyo, Japan; [4]Dept. of Neural Regeneration, Fukushima Med. Univ., Fukushima, Japan; [5]Dept. of Neurogy, Jikei Univ., Tokyo, Japan

Abstract: Background: Patients with dopamine deficiency is generally considered to exhibit slowed pace of internal clock, which is alleviated by intake of dopaminergic drug, as reported in patients with Parkinson's disease (PD). In contrast, some recent studies suggest accelerated rather than slowed internal clock in PD under some task conditions, which in turn correlates with dopamine deficiency as indexed by dopamine transporter imaging. We investigated the speed of internal clock using the temporal bisection task and temporal production/reproduction tasks, and correlated the performances of these tasks with the clinical severity and dopamine transporter imaging of the patients. Methods: Subjects were 21 PD patients (12 males, 9 females, age: 70.8±9.8), 5 patients with progressive supranuclear palsy (PSP; 4 males, 1 females, age: 75.8±5.5) and 20 age-matched normal subjects (10 males, 10 females, age: 73.0±6.8). Severity of Parkinsonism was assessed by the UPDRS motor score. In the temporal bisection task, the subjects judged the duration of successively presented tones as being closer in duration to either of the two formerly presented standard tones (400ms and 1600ms, or 2s and 8s) by pressing one

of two buttons (corresponding to "short"and "long"). In the production task, subjects produced time intervals indicated by the figures presented on a screen (e.g., 1sec, 2sec) by making two successive button presses, so that the time interval between the two presses would be the same as that indicated by the figure. In the reproduction task, subjects reproduced the time interval indicated by the duration of a circle presented on the monitor screen (1-8s) by making two successive button presses in a similar manner. Results: Duration of bisection of PD patients but not PSP patients was prolonged relative to normal subjects, which correlated mildly with the UPDRS motor score for the 2s and 8s bisection task, but not for the 400ms and 1600ms bisection task. Produced duration was prolonged in PD patients relative to normal subjects, but not in PSP patients. Reproduced time was comparably accurate for PD patients and normal subjects, but was significantly less accurate in PSP patients even if the severity of parkinsonism was taken into consideration. In PD, results of dopamine transporter imaging correlated weakly-moderately with the internal clock estimates of each patient (2s-8s bisection, production tasks). Discussion: Overall, the results indicated slower internal clock in the seconds range, but normal reproduction in PD patients. The production/reproduction in PSP patients were deteriorated compared with normal subjects and even PD patients.

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Poster

248. Human: Timing and Temporal Processing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 248.03/Z27

Topic: H.02. Human Cognition and Behavior

Support: NIH R01EY019466
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NIH R21EY028329
Brown University MRI Research Facility
Betty R.H. and James M. Pickett Fellowship Fund in Psychology

Title: Mechanisms of temporal perceptual learning through ^{1}H-magnetic resonance spectroscopy

Authors: *R. XU[1], E. G. WALSH[2], T. WATANABE[3], Y. SASAKI[4];
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Abstract: While the perceptual learning of time (TPL) constitutes one of the most profound demonstrations of neural plasticity in the brain, its underlying mechanism is poorly understood.

Using proton magnetic resonance spectroscopy (^{1}H-MRS) in conjunction with structural and functional connectivity analysis, we assessed whether TPL leads to neurochemical changes in the primary sensory and/or association cortices. Subjects (ages: 21 to 28 years) were trained over five days on an adaptive, two-interval auditory discrimination task. During the first and last day, we measured concentrations of glutamate and γ-aminobutyric acid (GABA) from the primary auditory (A1) and inferior parietal cortices (IPC). The relative ratio of excitatory-to-inhibitory neurotransmitters (E/I ratio) serve as a proxy for neural plasticity in the brain. Over five days of training, we observed a dissociable effect of task-related processing on the E/I ratios of the A1 and the IPC. Specifically, TPL was associated with a significant *increase* in E/I ratio of the IPC, and a *decrease* in E/I ratio of the A1, relative to baseline. This difference suggests that during initial stages of learning, the A1 and IPC may play complementary roles in interval learning. However, following five days of training, there was no difference in the E/I ratio changes in the two cortical areas compared to baselines. This is consistent with the hypothesis that TPL acts to consolidate a learning state and reorganize sensory and association cortices through shifts in the neurochemical balance between excitatory and inhibitory neural processing. As a result, performing a well-learned task no longer affects the overall degree of plasticity in a system as compared with initial stages of learning. Our findings present an initial exploration into the neurochemical processes that underlie the perceptual learning of time and provide grounds for constructing a unifying framework of perceptual learning across modalities.

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Poster

248. Human: Timing and Temporal Processing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 248.04/Z28

Topic: H.02. Human Cognition and Behavior

Title: Post-interval evoked potentials in temporal information processing

Authors: *E. ÖZOĞLU, R. THOMASCHKE;
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Abstract: Electrophysiological research on timing has traditionally focused on properties of contingent negative variation (CNV). Yet, a recent study proposed N1P2 as a novel electrophysiological marker to understand timing behavior, using a modified version of the temporal generalization task. The current study aimed at generalizing the applicability of this timing marker to a broader range of timing tasks while elucidating the cognitive differences between these tasks. To this end, we conducted a within-subjects study comparing a traditional temporal generalization task with a temporal bisection task. All participants (N = 39, 10 male, 35 right-handed) completed both tasks with simultaneous EEG recordings. The order of the tasks

was counterbalanced across participants. The analysis focused on two event-related potentials (ERP): interval onset-locked CNV latency, and interval offset-locked N1P2 amplitude. CNV results showed an unchanging peak latency for the generalization task, but a duration-based change for the bisection task. In the generalization task, the constant peak latency, irrespective of the current interval, can be assumed to correspond to timing of the target interval. N1P2 amplitude results were inconclusive for the generalization task, whereas a linear relationship between duration and amplitude was found in the bisection task. However, when considered together with the CNV results, this finding further supports the claim that N1P2 represents standard-probe mismatch. As the CNV was observed to increase from medium to long intervals (not timing a central reference anymore), the mismatch between the CNV and the current probe stayed low. These results partially supported existing findings concerning the generalization task and open up new questions about the neural foundation of timing in the bisection paradigm. Taken together with evidence from previous research, our findings indicate that N1P2 can indeed be regarded as a novel marker for neural timing; however, its interpretation might be more subtle than previously suggested.

Disclosures: E. ÖzoĞlu: None. **R. Thomaschke:** None.

Poster

248. Human: Timing and Temporal Processing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 248.05/Z29

Topic: H.02. Human Cognition and Behavior

Title: Accuracy and precision of stimulus timing and reaction times with Unreal Engine and SteamVR

Authors: *M. WIESING[1], G. R. FINK[2,3], R. WEIDNER[4,5];
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Abstract: The increasing interest in Virtual Reality (VR) as a tool for neuroscientific research contrasts with the current lack of established toolboxes and standards. In several recent studies, game engines like Unity [1] or Unreal Engine [2] were used [3, 4]. It remains to be tested whether these software packages provide sufficiently precise and accurate stimulus timings and time measurements that allow inferring ongoing mental and neural processes, which is, however, essential in neuroscience.
This project aimed at measuring the precision and accuracy of the timing mechanisms of Unreal Engine 4 and SteamVR [5] in combination with the HTC Vive VR system [6]. In a first experiment, we observed that stimuli were presented with highly accurate stimulus timings and

high precision in the range of 0.31 ms. In contrast, in a second experiment, we observed highly variable reaction time measurements with inaccurate stimulus onset measurements resulting in a mean measurement error of +55 ms and imprecise response time measurements in the range of 12 ms, when using the built-in timing procedures.
Hence, a new method was developed to provide precise and accurate reaction time measurements with Unreal Engine and SteamVR. Instead of measuring reaction times in Unreal Engine directly, the measurement part was outsourced to a background application. Timing benchmark results illustrated that the newly developed method allowed highly accurate reaction time measurements with a mean measurement error of 1.5 ms.
Overall, the present results indicate that the HTC Vive together with Unreal Engine and SteamVR can achieve high levels of precision and accuracy for stimulus timing. Furthermore, our newly developed method allows precise and accurate time measurements with Unreal Engine and SteamVR and can be used to obtain reliable timing parameters that not only allow accurate reaction times measures but also provides time measures that can be used in combination with time-sensitive functional measures.

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Disclosures: M. Wiesing: None. **G.R. Fink:** None. **R. Weidner:** None.

Poster

248. Human: Timing and Temporal Processing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 248.06/Z30

Topic: H.02. Human Cognition and Behavior

Title: Musical expertise generalizes to superior temporal scaling in a Morse code tapping task

Authors: *M. SLAYTON[1], J. L. ROMERO-SOSA[2], K. S. SHORE[1], D. V. BUONOMANO[3], I. VISKONTAS[4];
[1]San Francisco Conservatory of Music, San Francisco, CA; [3]Dept Neurobiol, [2]UCLA, Los Angeles, CA; [4]Univ. of San Francisco, San Francisco, CA

Abstract: A good musician must be able to keep accurate time across different tempi, adjusting to the demands of a conductor or musical partner. This skill is beneficial not just to musicians, however, but to anyone who needs to adapt a sequence of motor actions to changing environmental conditions. Therefore, a fundamental feature of temporal processing is the ability to produce motor patterns at different speeds. Here, we quantified temporal scaling and timing precision in musicians and non-musicians as they learned to tap a Morse Code sequence using their fingers. We found that musicians performed better than non-musicians both in terms of overall timing precision and in their ability to temporally scale--i.e., to reproduce the learned Morse code pattern accurately at faster and slower speeds. Interestingly, both musicians and non-musicians exhibited the Weber-speed effect: that is, absolute temporal precision improved when producing patterns at higher speeds. These results are consistent with previous findings indicating that neural mechanisms underlying timing improve with expertise, but suggest for the first time that the ability to generate the same motor patterns at different speeds also improves with extensive practice.

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Poster

248. Human: Timing and Temporal Processing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 248.07/Z31

Topic: H.02. Human Cognition and Behavior

Support: KAKENHI

Title: Dissociation of sensory from motor related signals for rhythm perception in the primate striatum and deep cerebellar nuclei

Authors: *M. KAMEDA[1], M. TANAKA[2];
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Abstract: Both the striatum and cerebellum are known to be involved in rhythm processing. We previously found that neurons in the caudate nucleus (*SfN abstr*, 2018) and the cerebellar dentate nucleus (*J Neurosci*, 2013) exhibited periodic firing modulation in monkeys attempting to detect a single omission of isochronous visual stimuli (missing oddball paradigm). To clarify their

functional difference, we further examined neuronal activity in these subcortical structures during the modified version of the missing oddball task that spatially dissociated sensory from motor components. In this task, the locations of repetitive visual stimulus and saccade target were independently selected from either visual hemifield in each trial, thus presenting four different combinations of stimulus and target locations.So far, we have recorded from a total 56 neurons (28 caudate and 28 dentate nuclear neurons) that exhibited periodic firing modulation during fixation. Neurons in the cerebellar dentate nucleusshowed a greater directional modulation for the repetitive visual stimulus that averaged 1.8-fold of the magnitude of directional modulation for saccade target (paired t-test, $P< 0.05$). In contrast, while some neurons in the caudate nucleus exhibited a greater directional modulation for saccade target than the repetitive visual stimulus, there was no significant bias of directionality for sensory or motor componentsin the population as a whole(paired t-test, $P= 0.40$). For caudate neurons, we also found that the directional modulation of baseline activity at the time of stimulus omission tended to be enhanced for saccade target (1.4-fold of directional modulation for repetitive stimulus), whereas the difference again did not reach the significant level (paired t-test, $P= 0.07$). When electrical microstimulation (50 μA)was delivered just before the stimulus omission to the recording sites in the caudate nucleus, the median of detection latency was significantly shortened in all stimulus conditions (paired t-test, $P< 0.05$). The amount of changes in latency significantly differed between saccade directions (18.4 ms, two-way ANOVA, $P< 0.05$), but not between repetitive stimulus locations (2.9 ms,$P= 0.99$). Taken together, these results suggest that during keeping track of repetitive stimulus timing, neurons in the striatum might represent periodic motor preparation, while those in the cerebellum might predict timing of each sensory event.

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Poster

248. Human: Timing and Temporal Processing

Location: Hall A

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Program #/Poster #: 248.08/Z32

Topic: H.02. Human Cognition and Behavior

Support: Wellcome Trust
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Leonard Wolfson Centre for Experimental Neurology
Medical Research Council UK
NIHR UCLH Biomedical Research Centre

Title: Structural neuroanatomy of temporal experience in dementia

Authors: *M.-C. REQUENA-KOMURO, C. R. MARSHALL, R. L. BOND, L. L. RUSSELL, C. GREAVES, K. M. MOORE, E. BENHAMOU, H. SIVASATHIASEELAN, J. L. AGUSTUS, C. J. D. HARDY, J. D. ROHRER, J. D. WARREN;
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Abstract: Spatiotemporal disorientation, mainly attributed to hippocampal atrophy, is a core feature of typical amnestic Alzheimer's disease (AD). Abnormalities of subjective time awareness have also been described in patients with frontotemporal dementia (FTD), notably behavioural variant FTD (bvFTD) and semantic dementia (SD). Here, we used a customised clinical questionnaire to probe changes in subjective time awareness in patients with major FTD syndromes (n=71), typical AD (n=28) and the major language-led AD variant syndrome, logopenic progressive aphasia (LPA; n=9), relative to healthy older individuals (n=32). Three major domains of temporal awareness were examined: the mental timeline (scheduling and ordering of events), mental timekeeping (clockwatching and temporal rigidity), and mental time travel (propensity to relive personal past events). A logistic regression model including diagnosis, age, gender, and disease severity as regressors was used to compare the proportion of individuals exhibiting each temporal awareness symptom between diagnostic groups. Neuroanatomical correlates were assessed using voxel-based morphometry of patients' brain MRIs following a standard protocol. Syndromic signatures of altered temporal awareness were identified. Patients with typical AD, LPA, and bvFTD were most prone to exhibit mental timeline difficulties, while patients with bvFTD and SD were most prone to exhibit abnormal mental timekeeping. Patients with bvFTD were also most inclined to relive past events. Grey matter associations were found for each time symptom within pre-specified anatomical regions of interest across the entire patient cohort and for particular syndromic groups. In line with the prevailing view that temporal processing engages distributed brain networks, neuroanatomical correlates of altered temporal awareness were extensive, with a particular focus on precuneus for mental timeline difficulties, and temporal polar cortex for mental timekeeping. Propensity to relive past events was positively associated with relatively preserved grey matter in a distributed left-sided fronto-temporo-parietal network. Taken together, these findings have implications for understanding the pathophysiology of temporal processing in neurodegenerative disease and potentially, the development of novel temporal biomarkers.

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Poster

248. Human: Timing and Temporal Processing

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Topic: H.02. Human Cognition and Behavior

Support: National Natural Science Foundation of China 81171019
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Title: Spatiotemporal dynamics of the processing of lexical information in word recognition revealed by intracerebral potentials

Authors: *Y. LIU[1], G. SHI[2], M. LI[1], Y. GUAN[3], Z. HAN[1];
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Abstract: One of the fundamental questions in language neuroscience is how the human brain processes the lexical information. It has been found a neural network which supports lexical information processing during word recognition (Paul et al., 2015; Lee et al., 2010; Wei et al., 2016). However, it is poorly understood the exact temporal and spatial distribution of this processing in the brain network. To address this issue, we collected stereoelectroencephalography (SEEG) data in 17 Chinese native epilepsy speakers with the implantation of 1393 electrodes in the left hemisphere, when the patients performed a lexical decision task (including 150 real Chinese characters and 150 Chinese non-characters). We examined when and where the lexical effect (i.e., the difference between non-characters and real characters) appeared in the high gamma-band activity (GBA) (60 HZ and 140 Hz) of SEEG signals. The earliest lexical effect (GBA signal was stronger for non-characters than for real characters; i.e., non-characters > real characters GBA) was observed in the occipital lobe (around 167 ms). Then, the reversed effect (real characters > non-characters GBA) appeared in the superior occipital lobe (around 136 ms), parietal lobe (around 332 ms) and inferior frontal gyrus (around 409 ms). Finally, the lexical effect was again detected in the middle frontal gyrus (around 515 ms). These findings highlight the ventral and dorsal pathways to collaboratively underlie the recognition of lexical information.

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Poster

248. Human: Timing and Temporal Processing

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Program #/Poster #: 248.10/Z34

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01-GM103894

Title: Temporal circuit of macroscale dynamic brain activity supports human consciousness

Authors: *Z. HUANG[1], J. ZHANG[2], A. G. HUDETZ[1];
[1]Dept. of Anesthesiol., Univ. of Michigan, Ann Arbor, MI; [2]Dept. of Anesthesiol., Fudan Univ., Shanghai, China

Abstract: Background. The conscious brain explores a dynamic repertoire of distinct states by constantly shaping and re-shaping the network of momentarily active and interacting brain regions. Clinical and preclinical investigations suggested that sparsification of the state repertoire may contribute to loss of consciousness in both neurological and anesthetized subjects but a unitary hypothesis for the critically involved brain regions or networks has not emerged. **Methods.** Using fMRI, we investigated the common and specific spatiotemporal characteristics of brain activity in human participants who were rendered behaviorally unresponsive (presumably unconscious) by different pharmacological interventions using either propofol or ketamine anesthesia, as well as in patients diagnosed with unresponsive wakefulness syndrome (UWS) as compared to healthy, conscious participants as controls. Unsupervised learning (k-means clustering) approach was applied to imaging data from all 98 participants. **Results.** We determined eight spatially distinct co-activation patterns (CAPs) at single-volume temporal resolution. Two of the CAPs with high anatomical segregation and anti-phasic activation were identified as the default-mode (DMN) and dorsal attention (DAT) networks; the temporal prevalence of the two CAPs was suppressed in both propofol and ketamine anesthesia and in UWS. Specific CAP changes included an increased prevalence of anti-phasic activation between visual (VIS) and ventral attention (VAT) networks with propofol, and an increased prevalence of global network activity (GN) with ketamine. UWS patients shared the latter two effects. Furthermore, in the conscious state, the CAPs followed structured temporal transition trajectories with reciprocal "source-target" relationships between CAPs. In contrast, the DMN and DAT were estranged from those trajectories that were instead monopolized by VIS, VAT, and GN during the unresponsive states. **Conclusions.** The momentary give-and-take relationship between DMN and DAT are embedded in a dynamic temporal "circuit" among available brain states in the conscious brain. The estrangement of DMN and DAT from the temporal circuit underlie pharmacological and neurological behavioral unresponsiveness.

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Poster

248. Human: Timing and Temporal Processing

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Topic: H.02. Human Cognition and Behavior

Support: NS092079
NS105839

Title: Distinct roles of alpha- and beta-band oscillations in temporal anticipation for perception or action in different modalities

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[1]Univ. of California, Berkeley, Berkeley, CA; [2]Psychiatry and Behavioral Sci., Stanford Sch. of Med., Palo Alto, CA; [3]Univ. California, Berkeley, CA

Abstract: The brain can anticipate when an event should occur based on temporal regularities in the environment, and allocate resources to facilitate perception and motor preparation. Electrophysiologically, temporal expectations are associated with amplitude suppression in the alpha band (8-13 Hz), beta band (15-25 Hz) or both, at the time of the expected event. It is not clear if these spectral modulations reflect general predictive timing computations, or mediate specific aspects of temporal expectations. For example, they could reflect preparation to a stimulus in a particular modality, or preparation of the appropriate response given a stimulus. To address this question, we measured EEG while human participants conducted a temporal prediction "ready-set-go" task. The time between Ready and Set was either 700 or 1700 ms. The time between the Set and Go (defined as the target) was usually identical; thus, participants could anticipate the onset of the target (valid trials). However, the target occurred at the non-cued interval (e.g., 1700 when expecting 700 ms interval) on a small number of trials (invalid). Participants completed four conditions in separate blocks. In two blocks, target were visual and in two blocks, auditory. Crossed was this was whether the target necessitated pure sensory processing (non-speeded classification of orientation or pitch in visual and auditory blocks, respectively), or also motor preparation (speeded response upon target detection). Behavioral performance was facilitated for valid compared to invalid targets (decreased RT on detection task and increased d-prime on classification task) for both visual and auditory targets, confirming formation of temporal expectations. In the EEG analysis, we focused on the comparison of trials in which the target was expected at 700 ms relative to 1700 ms. Alpha band activity was suppressed at occipital sites when anticipating a visual target, regardless of preparation goal. However, there was no suppression at these sites when anticipating an auditory target. Beta band activity was suppressed in central-parietal sites when the task emphasized motor preparation, regardless of whether the target was visual or auditory. Beta activity was also suppressed in the sensory preparation task when the target was auditory, but not when the target was visual. Taken together, these results indicate that alpha and beta suppression do not reflect general mechanisms of temporal expectation. Instead, these spectral modulations are involved in specific aspects of temporal expectation, alpha suppression in visual preparation, and beta suppression in audio-motor preparation.

Disclosures: **A. Breska:** None. **K.T. Duberg:** None. **R. Ivry:** None.

Poster

248. Human: Timing and Temporal Processing

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Topic: H.02. Human Cognition and Behavior

Support: EU-M-GATE 765549

Title: Effects of order on episodic memory of event times

Authors: *M. NAIM, M. KATKOV, M. V. TSODYKS;
Weizmann Inst. of Sci., Rehovot, Israel

Abstract: How do people remember the time of an event? The nature of time encoding and decoding is a fundamental open question. Episodic memory for time could employ two different processes (i) encoding of the absolute time of an event, (ii) encoding of its relative order compared to other events. To study the relative importance of these two processes and interactions between them, we performed a set of experiments in which one or two words were presented visually at a specific time during a certain interval, after which participants were asked to report the time of occurrence of a given word by moving a circle on a sliding bar. The results show that when a single word is presented, the distribution of reported times is quite wide, indicating that even a memory for a single event is unreliable. Moreover, we found that when two words are presented, the relative order among them strongly affected the memory for the time of each of them. In particular, for the same presented time, the reported time of the event was much later for the second word compared to the first one. The amplitude of this effect was about a quarter of the whole time interval, independently on the absolute presentation time. To explore the computational underpinnings of the observed effects, we developed a Bayesian theory of time representation for multiple events that combines absolute and relative time information contained in memory. The main assumption of the model is that memory for relative timing is more robust and hence contributes more to decoding. The model broadly compatible with the experimental data, in particular in terms of the effect of order on absolute time reports. Our results suggest that people's memory for absolute time of events relies critically on encoded order of the events and not the opposite.

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Poster

248. Human: Timing and Temporal Processing

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Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 248.13/Z37

Topic: H.02. Human Cognition and Behavior

Title: The human brain tracks probability of temporal deviance in regular auditory patterns

Authors: M. D. FOLDAL[1], A. O. BLENKMANN[1], A. LLORENS[2], T. ENDESTAD[1,3], A.-K. SOLBAKK[1,4,3];
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Abstract: Human event-related potential (ERP) studies have shown that stimulus predictability is reflected in attenuation of early negativities such as the auditory N1. In terms of event timing, N1 is attenuated when tones occur in regular (isochronous) compared to randomly timed sound sequences. However, it is not clear whether predictability of event timing beyond the immediate temporal context is encoded in a similar manner. One aspect related to the larger temporal context is the likelihood of temporal violation, which could impact the overall predictability in repeating regular sequences. Further, little is known about the role of selective attention in predictive processing. In the current study, we investigated effects of temporal predictability defined by the larger temporal context, and whether encoding of this information happens irrespective of spatial selective attention.
The effects of temporal predictability and attention on auditory ERPs were investigated using a dichotic listening task. The task involved listening to tones presented as continuous isochronous streams, in which probability of temporal deviance (-90 ms interval deviance) varied in order to manipulate temporal predictability. Auditory streams with different presentation rate (600 vs. 800 ms) were presented simultaneously, but to separate ears. In order to manipulate spatial selective attention, participants got instruction on which ear to attend to at the beginning of each experimental block. They responded with a button-press to temporal deviants within the attended stream. EEG data were recorded from 34 human subjects. Mean auditory N1 amplitude was investigated using a repeated measures ANOVA with two within-subject factors; temporal predictability (high vs. low) and attention (attended vs. unattended). The results revealed attenuation of the N1 when temporal predictability was increased, specifically by reducing the probability of temporal deviance. No statistically significant interaction was found between temporal predictability and spatial selective attention. In line with previous studies our results suggest that stimulus predictability is encoded in early auditory processing, and that these effects extend to the encoding of temporal predictability defined by the larger temporal context. Our

data may even suggest that encoding of such complex temporal predictability happens without allocation of spatial selective attention.

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Poster

248. Human: Timing and Temporal Processing

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Program #/Poster #: 248.14/Z38

Topic: H.02. Human Cognition and Behavior

Title: Subjective time estimation in humans relates to endogenous neural oscillations and geometry

Authors: *E. M. MILLON[1], A. E. HADDAD[2], L. NAJAFIZADEH[2], T. J. SHORS[1];
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Abstract: We are constantly estimating time in our everyday lives, but neuroscientists know little of how the brain makes decisions about time. Here we evaluated temporal decision-making with the temporal bisection (TB) task while recording event-related potentials (ERPs) from electroencephalography (EEG). By asking people to judge the temporal length of visual stimuli presented on a computer screen, the task yields a measure of perceived subjective equality (or the bisection point), which is when they are 50% likely to guess the stimulus is long versus short. Humans tend to bisect at the arithmetic mean, but the response is heavily influenced by the temporal spacing of stimuli (e.g., Allan and Gibbon, 1991; Kopec and Brody, 2010). EEG signals were filtered into three frequency bands: delta (1-4 Hz), theta (4-8 Hz) and alpha (8-16 Hz) because they revealed a well-timed response around the bisection point. These three rhythms have been tied to working memory performance. Alpha rhythms are implicated in sustained attention, theta and delta moreso in spatiotemporal characteristics of incoming information (Sadaghiani et al., 2016; Williams et al., 2019; Johnson et al., 2018). Less is known about how these rhythms relate to temporal decision-making. Nine right-handed adult women (n=9) were initially trained with two durations (400-ms or “short”, and 1600-ms or “long”). After learning to distinguish the two anchor stimuli, five probe stimuli were presented at intermediate durations (spaced every 200-ms), as well as the anchors themselves. Participants classified stimuli as being “short” (closer in time to 400-ms) or “long” (closer in time to 1600-ms) with a button press. EEG and ERP recordings were temporally segmented using the source-informed algorithm (Haddad et al., 2019), which allowed us to detect state transitions in the brain. We interpreted the longest segments as indicators of sustained functional, decision-making processes in the brain. These responses were filtered to visualize delta, theta, and alpha rhythms. On average, the longest

segment within the delta band occurred at 690-ms (SE = 55-ms), within the theta band at 798-ms (SE = 69-ms), and within the alpha band at 923-ms (SE = 46-ms). The mean behavioral bisection point was 784-ms (SE = 48-ms). Thus, collectively across bands, the neural response was closer to the behavioral bisection point and the geometric mean (800-ms), as opposed to the arithmetic mean (1000-ms). These data suggest that estimating time is accompanied by neural biomarkers in delta, theta and alpha bands that serve to discriminate between the temporal length of short and long stimulus durations.

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Poster

248. Human: Timing and Temporal Processing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 248.15/Z39

Topic: H.02. Human Cognition and Behavior

Support: European Union Future and Emerging Technologies grant (GA:641100) TIMESTORM

Title: An action-based predictive processing model of episodic memory explains time perception in the past, present and future

Authors: *Z. FOUNTAS[1], A. SYLAIDI[2], K. NIKIFOROU[2], W. ROSEBOOM[3], A. SETH[3], M. SHANAHAN[2];
[1]Inst. of Neurol., Univ. Col. London, London, United Kingdom; [2]Dept. of Computing, Imperial Col. London, London, United Kingdom; [3]Sackler Ctr. for Consciousness Sci., Univ. of Sussex, Brighton, United Kingdom

Abstract: Despite being a fundamental dimension of experience, the mechanism of the human brain that generates the perception of time, both prospectively and retrospectively, remains unknown. In this work, we provide a novel explanation of how human time perception might be accomplished, based on sequential, but non-temporal, episodic memory organization. We propose that the process of episodic memory formation is driven by the rate of perceptual change which creates the inner sense of duration in current experiences, while the processes of episodic memory recall and imagination, enriched by statistical semantic representations, drive duration judgements in the past and future respectively.To demonstrate this proposal, we designed an artificial neural system functionally similar to human visual processing, inspired by the concepts of hierarchical predictive coding, short-term plasticity, attention, episodic memory formation and recall. In addition, we conducted a large-scale experiment with 14,000 participants, to identify the effects of memory, cognitive engagement and stimulus context on the ability to report

durations. Estimates produced by our artificial system match the human reports, replicating key qualitative biases in all aforementioned cases. Furthermore, as the human brain actively samples given environments, the problem of time perception becomes highly intertwined with motor control. In previous work, duration estimates of a computational model were shown to better match human estimates when the model's input was constrained by human gaze data. The model presented here captures this effect by generating artificial gaze patterns driven by top-down prediction errors, which match human behaviour and underline the key importance of spatiotemporal attention. Overall, our approach provides a complete, working model of duration perception from real-life stimulus to estimation and from current experiences to mental time travel, opening new opportunities for investigating the foundations of this central aspect of human experience.

Disclosures: Z. Fountas: None. **A. Sylaidi:** None. **K. Nikiforou:** None. **W. Roseboom:** None. **A. Seth:** None. **M. Shanahan:** None.

Poster

248. Human: Timing and Temporal Processing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 248.16/Z40

Topic: H.02. Human Cognition and Behavior

Title: The timing of music appraisal judgments

Authors: *S. SPIVACK[1], A. POINCOT[1], S. E. MCCLELLAND[1], D. TOSSAVAINEN[1], L. E. CRANMER[1], G. IENNER[1], P. WALLISCH[2];
[2]Ctr. Neural Sci., [1]New York Univ., New York, NY

Abstract: We are interested in the timing of music appraisal judgments. We explored this question by asking a high-powered sample of participants to appraise a large and representative corpus of short music clips from a diverse range of genres and time periods. We found that the reaction time of appraisal judgments varies as function of familiarity, valence and magnitude, as well as specific emotional responses. We explain our results in terms of a diffusion-to-bound model in which negative judgments are faster, consistent with a risk-avoidance model of decision making.

Disclosures: S. Spivack: None. **A. Poincot:** None. **S.E. McClelland:** None. **D. Tossavainen:** None. **L.E. Cranmer:** None. **G. Ienner:** None. **P. Wallisch:** None.

Poster

248. Human: Timing and Temporal Processing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 248.17/Z41

Topic: H.02. Human Cognition and Behavior

Support: NSF EPSCoR Award Number 1632738

Title: High-order brain network dynamics support cognitive processing

Authors: *L. L. OWEN, J. R. MANNING;
Dept. of Brain and Psychological Sci., Dartmouth Col., Hanover, NH

Abstract: Dynamic interactions between brain structures underlie our thoughts. We developed a model of neural dynamics that takes in a feature timeseries and outputs estimated first-order network dynamics (i.e., dynamic functional correlations), second-order network dynamics (reflecting homologous networks that dynamically form and disperse), and higher-order network dynamics (up to tenth-order dynamic correlations). After validating our model using synthetic data, we applied the approach to an fMRI dataset collected by Simony et al. (2016) as participants listened to an audio recording of a story, as well as scrambled versions of the same story (where the scrambling was applied at different temporal scales). We trained classifiers to take the output of the model and decode the timepoint in the story (or scrambled story) that the participants were listening to. We found that, during each of the listening conditions in the experiment, classifiers that incorporated second-order correlations yielded consistently higher accuracy than classifiers trained only on lower-order patterns. (Incorporating higher-order correlations did not further improve decoding accuracy.) By contrast, these higher-order correlations were not necessary to support (minimally above chance) decoding during a control rest condition. This suggests that the cognitive processing that supported the listening conditions involved second-order (or higher) network dynamics.
In a second series of analyses, we applied dimensionality reduction algorithms to the activity patterns in each experimental condition. Specifically, we sought to understand the "dimensionality" of the neural patterns that were sufficient to decode participants' listening times (or approach was similar to that of Mack et al. 2017). We found that even low-dimensional embeddings of the data were sufficient to accurately decode listening times from the intact story recording, whereas finer temporal scramblings of the story required higher-dimensional embeddings of the data to reach peak decoding accuracy.
Our work suggests that our thoughts arise from (at least) second-order neural dynamics, and the dimensionality of neural representations of our experiences changes according to how richly and/or deeply those experiences are processed.

Disclosures: L.L. Owen: None. **J.R. Manning:** None.

Poster

248. Human: Timing and Temporal Processing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 248.18/Z42

Topic: H.02. Human Cognition and Behavior

Title: Cognitive refractory state caused by spontaneous epileptic high frequency oscillations in the human brain

Authors: *S. LIU, J. PARVIZI;
Neurol. and Neurolog. Sci., Stanford Univ., Stanford, CA

Abstract: Epileptic brain tissue is often considered pathological and the optimal treatment of patients with uncontrollable seizures involves identifying the tissue and aiming for its complete resection. However, cognitive deficits ensuing such surgical resections, especially of non-lesional brain sites, remain of critical concern. Moreover, it remains unclear to what extent the epileptic tissue is capable of generating physiological responses to cognitive stimuli, and if so, how these responses are affected by ongoing spontaneous epileptic activity. In the current intracranial EEG study, we recruited six patients with intracranial electrode coverage in either visual association cortex (N=3) or in the medial temporal lobes (MTL, N=3) who participated in site-relevant cognitive experiments. We identified the epileptic focus in each patient and measured their spontaneous high frequency oscillations (HFOs) as well as stimulus-locked physiological responses in high frequency broadband (HFB) range and explored their interaction and behavioral correlates. Consistently in all subjects, we found abundant normal physiological responses to relevant cognitive stimuli in the epileptic sites, but they were likely to be “seized” when spontaneous HFOs occurred approximately 850-1050ms before, till about 150-250ms after, the onset of relevant cognitive stimuli. Ongoing spontaneous HFOs in the MTL significantly impacted the subjects’ memory performance. Lastly, we report a computerized method on the basis of which pathological and physiological high frequency activities can be automatically differentiated. The SVM classifier successfully separated HFOs from HFBs using our proposed features, achieving an AUC value of 0.98. Our findings clearly suggest that non-lesional brain structures involved with epileptogenicity elicit normal physiological responses to cognitive stimuli and highlight a compelling mechanism for cognitive deficits in patients with focal epilepsy. We also offer clinicians a quantitative tool for differentiating pathological and physiological activities in epileptic sites and indirectly assessing their possible cognitive reserve function and approximating the risk of resective surgery.

Disclosures: **S. Liu:** None. **J. Parvizi:** None.

Poster

248. Human: Timing and Temporal Processing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 248.19/AA1

Topic: H.02. Human Cognition and Behavior

Support: JSPS KAKENHI Grant Number 16K12969
JSPS KAKENHI Grant Number 16H01866
JSPS KAKENHI Grant Number 19H01087

Title: Prior asynchrony causes a positive aftereffect on tactile synchrony judgement

Authors: *K. WIDJAJA[1], K. SAITO[2], K. KANNAGA[1], D. YOSHIOKA[3], Y. ITAGUCHI[1,2], M. MIYAZAKI[1,2,3];
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Abstract: The subjective time in humans adapts to prior experiences. In this study, we investigated the effect of prior synchrony/asynchrony on tactile synchrony judgment. Participants (n = 12) sequentially received two pairs of tactile stimuli across the hands: an adaptor stimulus pair and a test stimulus pair. The participants judged whether the test stimulus pair was synchronous or asynchronous. The stimulus onset asynchrony (SOA) for the adaptor stimulus pair (a-SOA) was -100, 0, or 100 ms. The SOA for the target stimulus pair (t-SOA) was -80, -30, -10, 0, 10, 30, or 80 ms. The interstimulus interval (ISI) between the adaptor and test stimulus pairs was 500, 1000, or 2000 ms. The participants completed 12 sessions (63 trials each) of the synchrony judgment task. As a result, they judged the test stimulus pair as 'asynchronous' with greater frequency when the adaptor stimulus pair was asynchronous (a-SOA = ±100 ms) compared to when it was synchronous (a-SOA = 0 ms). This indicates that a positive aftereffect occurred in tactile synchrony judgment. This effect appeared under the ISI conditions of 500 ms and 1000 ms, but it disappeared under the ISI conditions of 2000 ms. Moreover, there was no difference in the judgment rates among the ISI conditions when the adaptor stimulus pairs were synchronous (a-SOA = 0 ms), suggesting that the aftereffect occurred only when the adaptor stimuli were asynchronous. Thus, our results showed that prior asynchronous tactile stimuli caused a positive aftereffect on tactile synchrony judgment. This positive aftereffect was opposite to the lag adaptation observed in audiovisual synchrony judgment, but consistent with a prediction based on the optimal Bayesian estimation model.

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Poster

248. Human: Timing and Temporal Processing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 248.20/AA2

Topic: H.02. Human Cognition and Behavior

Support: NSERC Discovery Grant
VISTA Fellowship supported by the Canada First Research Excellence Fund

Title: Linear vector models of time perception accurately and specifically simulate temporal distortions associated with stimulus novelty and saccades

Authors: *A. H. GHADERI[1,2], J. D. CRAWFORD[1,2,3,4,5];
[1]Ctr. for Vision Res., [2]Vision Sci. to Applications (VISTA) Program, [3]Departments of Biol., [4]Psychology, [5]Kinesiology and Hlth. Sci., York Univ., Toronto, ON, Canada

Abstract: Subjective time is compressed during saccades, whereas it is dilated during presentation of a novel stimulus. Classical time perception models, such as the internal clock model (which suggests a pacemaker to create subjective time), cannot describe these apparent time distortions. Other models have been proposed to explain these individual effects, but there is no comprehensive model to explain both. Here, we used an empirical dataset that is collected in a related psychophysics study (an oddball/saccade paradigm) to compare the efficiency of three computational models 1) linear scalar (that used a linear scalar summation between inputs with two variable coefficients) and 2) linear vector models (that considered angles between inputs and used a vector summation between inputs) and 3) a neural network timing model (that employed a multilayers perceptron structure and a back-propagation learning algorithm). The dataset contains responses of 10 participants when they compared the duration of a test stimulus (200 ms) with duration of target (between 140 and 260 ms) during three different conditions: 1) saccade (S) (performing saccade 100 ms before target presentation), 2) novelty (N) (repetition of test stimulus before target) and 3) both saccade and novelty (S-N). The responses in the conditions S and N were used as inputs of models and outputs were responses in condition S-N. The results indicate that both vector and neural network models were able to find highly correlated outputs ($R>0.8$) with empirical data while the scalar model failed to find an association between inputs and outputs ($R<0.3$). At this point, both vector and neural network models complied with our experimental result that, although the time compression occurred in the saccade condition, this effect was diminished by novelty of stimulus. To test the specificity of these fits, we used a random shuffled version of original dataset. The neural network model performs high accuracy predictions ($R>0.8$) whereas the vector model was invalid ($R<0.3$). This shows that vector model predictions are both specific and accurate for both effects (perisaccadic

and stimulus novelty) and suggests that subjective time can be created as vector units of neural associations.

Disclosures: A.H. Ghaderi: None. **J.D. Crawford:** None.

Poster

248. Human: Timing and Temporal Processing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 248.21/AA3

Topic: H.02. Human Cognition and Behavior

Support: MOE Tier 1
NTU CoHASS Incentive Scheme
NTU URECA

Title: Temporal and spatial ensemble statistics are formed by distinct mechanisms

Authors: *H. XU[1], H. YING[2], E. BURNS[3], A. M. CHOO[1];
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Abstract: Our brains are capable of extracting a summary representation of the facial characteristics provided by a group of faces. To date, there has been a lack of clarity as to what calculations the brain is actually performing during this ensemble perception. For example, are faces in a scene computationally averaged together from their facial feature, or is an ensemble derived from the facial characteristics (e.g. these faces are unattractive)? Here we take advantage of the fact that a morphed face averaged from the facial feature of a group of faces is more attractive than the mean facial characteristics perceived on each face individually. If ensemble perception is performing facial feature averaging, then the adaptation aftereffects elicited by the morphed average face of a group of faces should be equivalent to those elicited by the group. By contrast, if ensemble perception reflects the averaging of facial characteristics, then the aftereffects should be distinct from those elicited by the more attractive morphed average. In support of the facial feature averaging hypothesis, we show that the adaptation aftereffects derived via temporal ensemble perception of a group of faces are equal to those produced by a morphed average face. In a follow up experiment, we show that these effects increase as a linear function of increasing attractiveness in the underlying group. Remarkably in our third experiment, we show that spatial ensemble perception is not equal to temporal ensemble perception, but instead appears to be derived from the facial characteristics judgements attributed to the faces. In the final experiment, the spatial-temporal facial streams, combining Experiment 2&3, showed a pattern which is similar to temporal rather than spatial ensemble, suggesting that the observed results are not caused by variances in experimental designs but by different

averaging mechanisms. We have therefore shown for the first time that temporal and spatial ensemble statistics arise from distinct mechanisms that produce qualitatively different perceptual outcomes.

Disclosures: H. Xu: None. **H. Ying:** None. **E. Burns:** None. **A.M. Choo:** None.

Poster

249. Human Social Cognition: Behavior, Mechanisms, and Disorders II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 249.01/AA4

Topic: H.02. Human Cognition and Behavior

Title: Cortical functionality in homosexual and heterosexual men in response to visual sexual stimuli

Authors: *C. AMEZCUA, D. LOZANO-MARTÍNEZ, M. HERNÁNDEZ-GONZÁLEZ, E. HERNÁNDEZ-ARTEAGA, M. GUEVARA;
Univ. De Guadalajara, Guadalajara, Mexico

Abstract: The perception of stimuli with sexual content depends on sexual preference. Since several cortical areas participate in the processing of sexual stimuli, this study characterized the absolute power and electroencephalographic correlation among prefrontal, temporal and parietal areas in men with different sexual preference while observing erotic videos with homosexual and heterosexual content. The homosexual group (HOG) rated the homosexual video (HOV) as more pleasurable, with higher general and sexual arousal than the heterosexual video (HEV). This group presented lower absolute power of theta band in the right temporal cortex and higher right fronto-temporal correlation of beta band when observing the HOV, likely associated with the pleasant valence and the global, or contextual, processing of the stimulus with sexual connotation of their preference. The heterosexual group (HEG) that rated the HEV as pleasant, presented a higher left fronto-temporal correlation of beta band while watching the HEV with a lower right fronto-parietal correlation of theta band during observation of the HOV, could be associated with greater attention and cognitive processing, focused on the specific aspects of the stimuli. These results contribute to our knowledge of the cortical functionality involved in the processing of sexually-relevant stimuli and its possible association with sexual preference.

Disclosures: C. Amezcua: None. **D. Lozano-Martínez:** None. **M. Hernández-González:** None. **E. Hernández-Arteaga:** None. **M. Guevara:** None.

Poster

249. Human Social Cognition: Behavior, Mechanisms, and Disorders II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 249.02/AA5

Topic: H.02. Human Cognition and Behavior

Support: Grant-in-Aid for Scientific Research(B) (18H03304)

Title: Information flow among brain networks in depressive states by using transfer entropy and phase synchronization with human EEG

Authors: *M. KAWASAKI, K. AIBA, E. MIYAUCHI;
Univ. of Tsukuba, Tsukuba, Japan

Abstract: Recent studies have revealed a strong relationship between the deficits of the brain networks and major depression disorder (MDD). The brain networks include default mode networks as well as the sensory-motor active brain networks. The brain networks are evaluated by the simultaneous activations among several brain areas. For example, the default mode networks show simultaneous increases during the resting state and decreases during cognitive tasks. However, it is not clear about the directions of information flows among the brain areas in relation to the MDD symptoms. To address the issues, we tried to evaluate the information flows by using the phase synchronization and transfer entropy of the human electroencephalogram (EEG) data which was recorded during the resting states. First, we analyzed the EEG data of the MDD patients and evaluated the transfer of the EEG phase resetting with the transcranial magnetic stimulation (TMS) to the visual areas. The EEG alpha phase resetting was transferred from the visual and motor areas in the healthy participants or the remitted patients, whereas it was not observed in the MDD patients. The degrees of the information transfer were correlated with the MDD symptoms. Therefore, the deficits of the information transfer among the sensory-motor brain network reflected the MDD symptoms. Second, we analyzed the EEG data of the undiagnosed participants in the early stages of the depressive states and evaluated the transfer entropy of the EEG phases during the resting states with the auditory stimulus. The results showed that the beta-band transfer entropy from the auditory area to the parietal area was high as information flow among the default mode networks. These results suggest that the default mode network may already be altered during the early stages of the depressive states.

Disclosures: M. Kawasaki: None. **K. Aiba:** None. **E. Miyauchi:** None.

Poster

249. Human Social Cognition: Behavior, Mechanisms, and Disorders II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 249.03/AA6

Topic: H.02. Human Cognition and Behavior

Title: Cellular representations of human theory of mind

Authors: *M. JAMALI, B. GRANNAN, R. BÁEZ-MENDOZA, Z. WILLIAMS; Massachusetts Gen. Hospital/Harvard Med. Sch., Boston, MA

Abstract: A foundation of human social cognition is our capacity to attribute mental states to others and to recognize that others may have different internal states or beliefs than our own. This ability, often termed 'theory of mind', plays a vital role in social behavior and is broadly disrupted in psychiatric conditions such as autism spectrum disorder, bipolar disorder and schizophrenia. The human prefrontal cortex has been implicated in inferential processing and the ability to reason about others, and is broadly connected with limbic and temporal-parietal areas thought to be involved in social behavior. To date, however, the underlying computations formulated by individual neurons during this complex cognitive process in the human brain remains unknown. Here, we use the false-belief behavioral task and acute single-neuronal recordings from the human dorsomedial prefrontal cortex (dmPFC), an area implicated in social cognition, to find cells that reflect core features describing the beliefs of other individuals. The subjects are presented with short narratives about real-world scenarios and are required to formulate an idea about the hidden state-of-mind of individuals in the narratives and demonstrate understanding of another's beliefs even when those beliefs differ from one's own. Across 11 participants, we recorded from a total of 212 dmPFC neurons, of which 20% responded selectively to another's beliefs and differentiated another's beliefs from the participant's own. Further, when modeled collectively, these neurons accurately predicted whether the other's beliefs were false (78±3% decoding accuracy) and reflected the other's implied awareness of events, together suggesting that they responded to the other's unique state. These neuronal predictions were robust to differences in belief context such as identity or location, but were represented by largely distinct populations of cells. Together, our study reveals neurons in the prefrontal cortex that reflect the inferred beliefs of other individuals and may function to support human theory of mind.

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Poster

249. Human Social Cognition: Behavior, Mechanisms, and Disorders II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 249.04/AA7

Topic: H.02. Human Cognition and Behavior

Support: NIH Intramural Research Program ZIAMH002920

Title: Neural mechanisms of shared social attention revealed by independent measures of eye movement typicality

Authors: *M. RAMOT, C. WALSH, G. E. REIMANN, A. MARTIN;
NIH/NIMH, Bethesda, MD

Abstract: Navigating our complex social world requires mechanisms of directed attention to allow us to select the most relevant information. Directors of Hollywood movies are particularly adept at manipulating the focus of our attention, using cinematic techniques to create a movie experience which is largely shared across viewers. In this study, we examine the underpinnings of this shared attention in a large cohort (60 participants). Participants first watched 24 short (14s) movie clips outside the scanner. These were chosen in a separate pilot study from a larger set of 60 movie clips, for eliciting the most consistent viewing patterns. However, even within these carefully selected movie clips, there was a range of individual eye movements, with some participants having more typical viewing patterns than others. We quantified the typicality of eye movements for each participant by calculating the Euclidean distance from the mean scan path of all other participants, averaged across all the frames and all the movies. We next quantified the typicality of the neural responses for each participant while watching a different 9 minute movie inside the scanner. We calculated the correlation of the timecourse of each voxel to the average timecourse of that voxel for all the other participants, giving us a measure of how typical (i.e. similar to the average) the neural responses to the movie were for that participant. Finally, we combined these two independent measures of brain and behavior, by conducting a whole brain search for voxels in which there was a correlation across participants of the typicality of the neural responses to the movie and the typicality of the eye movement patterns during the short movie clips shown outside the scanner. Our results reveal a network of social/language processing regions comprised of the superior temporal sulcus, left inferior frontal gyrus, anterior insula, as well as the hippocampus and left-lateralized caudate, for which eye movement typicality is strongly correlated with neural typicality in response to a movie (FDR corrected, $q<0.05$). These findings are consistent with the view that our shared social attention is underpinned by a network of social, language, and memory processing regions.

Disclosures: **M. Ramot:** None. **C. Walsh:** None. **G.E. Reimann:** None. **A. Martin:** None.

Poster

249. Human Social Cognition: Behavior, Mechanisms, and Disorders II

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Topic: H.02. Human Cognition and Behavior

Support: Scientific Research Network on Decision Neuroscience awarded to DVS [Subaward of NIH R24-AG054355 (PI Samanez-Larkin)]
College of Liberal Arts at Temple University (DVS)
NIH grant R21-MH116422 (DVS)

Title: Response to perceived fairness is associated with reduced connectivity within reward circuitry in older adults

Authors: *K. HACKETT[1], N. M. HENNINGER[2], V. KELLY[1], T. GIOVANNETTI[1], D. S. FARERI[3], D. V. SMITH[1];
[1]Dept. of Psychology, Temple Univ., Philadelphia, PA; [2]Temple Univ., Bensalem, PA; [3]Psychology, Adelphi Univ., Garden City, NY

Abstract: Understanding the behavioral and neural correlates of perceived fairness and equity in social interactions across the lifespan has wide ranging theoretical and practical implications. Researchers have studied this using the Ultimatum Game (UG) in which a Proposer offers to share a set amount of money with a Responder, who can choose to accept or reject the proposed split. Prior research in younger adults suggests that Responders tend to reject offers that are perceived to be unfair, but responses are affected by what they know about the Proposer; also, fair offers evoke activation in the striatum while rejection of unfair offers evokes activation in the insula and dorsolateral prefrontal cortex. However, it remains unclear whether the behavioral and neural correlates underlying responses to perceived fairness differ between older and younger adults. fMRI was obtained from thirty-two older (n=13, ages 65-80) and younger (n=19, ages 20-35) adults acting as the Responder in a block-design variant of the UG that included three types of anonymous Proposers: older adults, younger adults, and computers (visually depicted by cartoon emojis). On each trial, participants were presented a cue indicating proposer type, followed by a fair (35-50%) or unfair (5-20%) proposed division of $20. Both older and younger adults accepted significantly more fair vs. unfair offers across all proposer types. There were no significant main effects of age on response to fair vs. unfair offers, and no significant interactions with proposer type. However, an interaction between age group and proposer type in reaction time was trending towards significance (p=.067) such that fair offers from a computer elicited the slowest response in older adults and the fastest response in younger adults. fMRI analyses revealed that unfair blocks evoked increased activation in the temporoparietal junction in younger adults relative to older adults. Network psychophysiological interaction (nPPI)

analyses demonstrated that relative to older adults, younger adults showed increased connectivity between the ventral striatum and the ventral medial prefrontal cortex when playing against a human vs. computer proposer, regardless of offer type. Our results suggest that social and affective processes underlying perceived fairness/equity may differ across age groups. These findings may have important implications as we continue to learn about age-related changes in social cognition/closeness and vulnerability to financial exploitation.

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Poster

249. Human Social Cognition: Behavior, Mechanisms, and Disorders II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 249.06/AA9

Topic: H.02. Human Cognition and Behavior

Title: The effect of action contingency on social perception are prolonged after interaction with others

Authors: *Y. HAMAMOTO[1], Y. TAKAHARA[1], K. KAWATA[3], T. KIKUCHI[1], S. SUZUKI[2,3], R. KAWASHIMA[3], M. SUGIURA[3,4];
[1]Sch. of Med., [2]Frontier Res. Inst. for Interdisciplinary Sci., Tohoku Univ., Sendai, Japan; [3]IDAC, Tohoku Univ., Sendai, Japan; [4]Intl. Res. Inst. of Disaster Science, Tohoku Univ., Sendai, Japan

Abstract: Contingency in the sensory feedback to one's own action is considered to increase social perception such as intimacy. Neural evidence of the real-time effect of action contingency has been demonstrated during interaction with the partner, but not the prolonged effect after recognition of the partner (i.e., without action). Furthermore, it hasn't yet been examined if the effect is different between the animate and inanimate partners. Therefore, we examined the effect of action contingency on the social perception after interacting with animate and inanimate partners, and investigate neural activity related to this effect. Subjects were 31 healthy right-handed people. They underwent the fMRI experiment composed of two tasks: interaction task and evaluation task. In interaction task, subjects pressed a left or right button in response to the cue, which resulted in the presentation of a red square on the left and right, respectively, to the fixation cross. A face (F) or object (O) avatar placed above the cross changed the direction to the red square immediately after the button press (C: contingent) or made a similar movement at a random timing and to a random direction (N: non-contingent). After the interaction task, subjects performed evaluation task, in which each avatar was presented 1 sec without action and then subjects rated the sense of intimacy for each avatar using three questionnaire items. Each subject performed five sessions (Task), and the evaluation task was also performed before the first

session (Pre). We analyzed brain activity during all sessions in the evaluation task while subjects were looking at each avatar just before rating. We performed two-way ANOVA to investigate the main effect of Contingency [C vs. N] and Contingency x Animacy [F vs. O] interaction effect on intimacy change (Task - Pre). We found a significant main effect of Contingency, but the interaction was only marginally significant. Then we identified the brain activity corresponding to main effect of Contingency: left middle frontal gyrus (MFG), right inferior frontal gyrus (IFG) and bilateral lingual gyri (LiG) were significantly deactivated in C condition compared with N condition. We showed prolonged action contingency effect on social perception change and provided its neural evidence irrespective of the type of partner (i.e., animate or inanimate). We revealed the relationship between the increase in intimacy and the deactivation of MFG, IFG and LiG, and our result is consistent with previous findings suggesting these regions' activation is related to negative impression toward others.

Disclosures: Y. Hamamoto: None. **Y. Takahara:** None. **K. Kawata:** None. **T. Kikuchi:** None. **S. Suzuki:** None. **R. Kawashima:** None. **M. Sugiura:** None.

Poster

249. Human Social Cognition: Behavior, Mechanisms, and Disorders II

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Program #/Poster #: 249.07/AA10

Topic: H.02. Human Cognition and Behavior

Support: Fred B. Snite Foundation

Title: A systematic review of sex difference in human neonatal social orientation

Authors: L. KARSON[1], H. MINHAS[1], J. DAVIES[1], M. DHESI[1], J. PATEL[1], ***L. S. ELIOT**[2]; [1]Chicago Med. Sch., Rosalind Franklin Univ., North Chicago, IL; [2]Neurosci., Rosalind Franklin Univ. of Med. & Sci., North Chicago, IL

Abstract: Women score higher than men on measures of social cognition such as empathy and reading non-verbal cues. How early does this sex difference emerge? A frequent claim is that males are less "people-oriented" than females from birth, a difference hypothesized to be shaped by prenatal testosterone and to underlie higher male prevalence of autism spectrum disorders (Baron-Cohen et al., 2005). Sex difference in neonatal social orientation was not found by Maccoby & Jacklin in their large review, *The Psychology of Sex Differences* (M&J, 1974). To update this analysis and test the hypothesis of innate onset, we undertook a systematic review of sex difference in social sensitivity in human neonates.
We searched PubMed and PsychInfo using the terms [(face OR social) AND (perception OR recognition OR attention OR discrimination)] under the limits [human, birth to 1 month, English language]. We also hand-searched all article titles across the lifetime of 8 developmental

psychology journals plus all neonatal studies in M&J (1974). Studies were sorted according to dependent measure: 1) visual fixation time or preference for social stimuli, 2) “animate” versus “inanimate” orientation subscores on the Brazelton Neonatal Behavior Assessment Scale (NBAS), and 3) others, including imitation, high-amplitude sucking, and empathic crying.
A total of 27 studies reported on sex effects for social orientation in 1,683 neonates. Of these, it was possible to calculate effect sizes in 19 of the studies. A remaining 8 studies reported only qualitative results, with 7 stating “no significant sex differences” were found. Among the 17 visual fixation studies, 2 reported a significant difference favoring females, 1 reported a difference favoring males and 14 found no differences. Among the NBAS studies, 2 reported no sex difference in overall orientation score, 1 reported greater male orientation and 3 reported stronger female orientation to several stimuli, both animate and inanimate. Within the “other” category, females were found to imitate more, cry more, or suck more to social stimuli in 3 out of 4 studies.
In summary, collective evidence does not demonstrate a clear female advantage for social orientation or perception at birth. Weakness in existing data include: low sample sizes, insufficient quantitative reporting, and lack of experimenter blinding to infant sex. Future research should address the effect of global maturation on social perception as well as possible biological (e.g., neonatal gonadal hormones) and learned (e.g., gender-differentiated parenting) influences over social cognitive development in boys and girls.

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Poster

249. Human Social Cognition: Behavior, Mechanisms, and Disorders II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 249.08/AA11

Topic: H.02. Human Cognition and Behavior

Title: Social processing in autism spectrum disorders using a machine learning approach to visual stimulus segmentation

Authors: *G. E. REIMANN[1], M. RAMOT[1], C. WALSH[1], P. MCCLURE[2], F. PEREIRA[2], A. MARTIN[1];
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Abstract: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder, associated with reciprocal interaction and social communication difficulties. Among these impairments is poor eye contact modulation, which is detectable within the first few years of life and often continues into adulthood. Eye tracking technology, in conjunction with dynamic, naturalistic scenes,

provides unique insight on the complexities of eye movement patterns. One persisting challenge, however, remains linking accurate gaze locations with the sizable content derived from dynamic stimuli. The present study sought to evaluate ASD and typically developing eye movement patterns by utilizing a machine learning algorithm to generate objective labeling of naturalistic scenes. In this study, a deep neural network was trained on an existing image dataset, labeled for body parts and background (PASCAL-Part), then applied to assign labels to 24 novel and unlabeled movie clips (14s each, 9,360 frames total). Participants with ASD (n=37) and their age-matched typically developing (TD) controls viewed these Hollywood movie clips, each of which depicted a social interaction. Controls spent significantly more time looking at facial features than participants with ASD ($p < 0.001$). Participants with ASD looked significantly more outside the face, at other body parts, or at background objects compared to their matched TDs ($p < 0.001$). Behavioral data plays an integral role in understanding the brain, linking brain states to a measurable output. As we seek to explore intricacy of ASD gaze patterns, using machine learning for visual stimulus segmentation can advance the technical capability of eye movement analyses. Supplemented by these tools, researchers can utilize eye tracking to support diagnoses, assess progress of social trainings, and ultimately evaluate individual or population differences of social processing.

Disclosures: **G.E. Reimann:** None. **M. Ramot:** None. **C. Walsh:** None. **P. McClure:** None. **F. Pereira:** None. **A. Martin:** None.

Poster

249. Human Social Cognition: Behavior, Mechanisms, and Disorders II

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Program #/Poster #: 249.09/AA12

Topic: H.02. Human Cognition and Behavior

Support: KAKEN Grant 19K07803

Title: Being in a social majority enhances decision confidence and activates frontostriatal neuroarchitecture

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Abstract: Decision confidence, i.e., the internal perception of judgment accuracy, enables adaptive behavior in changing natural and social environments. However, it remained largely unknown how social information impacts decision confidence. Here, we addressed this open question by investigating how decision confidence changes as a function of whether a majority or minority of others made the same decision. In the fMRI scanner, participants engaged in

commonly used binary decision tasks consisting of a basic face versus house discrimination or a random dot motion discrimination. After each decision, in one half of the trials, a pie chart indicated the proportion of people who made the same decision as the participant. We varied this social information between trials by showing one out of eight different proportions irrespective of participant accuracy. In the other half of the trials we presented no social information (no-influence). Next, the participant provided a confidence rating regarding their present decision. We compared three conditions (social majority, social minority and no-influence) with a one-way ANOVA. Behaviorally, decision accuracy in the discrimination tasks did not differ across conditions. However, decision confidence significantly increased in the social majority condition and decreased in the social minority condition compared to the no-influence condition. In the brain, at the time of the confidence rating, ventromedial prefrontal cortex activity increased parametrically with confidence, more so in the no-influence than the majority and minority conditions. Caudate and mid-cingulate regions showed stronger confidence coding in majority than minority and no-influence conditions, irrespective of the decision being correct or incorrect. Moreover, dorsolateral prefrontal cortex and anterior cingulate cortex were significantly more active in the majority condition compared to the minority condition only when the decision later turned out to be incorrect. These results suggest that social information influences decision confidence and frontostriatal regions underpin this change in confidence for conventionally wrong and right decisions.

Disclosures: **J. Fujiwara:** None. **P.N. Tobler:** None. **S. Eifuku:** None.

Poster

249. Human Social Cognition: Behavior, Mechanisms, and Disorders II

Location: Hall A

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Program #/Poster #: 249.10/AA13

Topic: H.02. Human Cognition and Behavior

Title: Habituation in subjects with and without suicide attempt

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Abstract: Habituation is related to learning and adaptation. It shows clear reduction in amplitude of cognitive waves (ERPs). The serotonergic system is involved in habituation and a deficit of 5-HT metabolites has been related to self-injury and suicide. Although ERPs has been used as indicators of suicide risk, there are no studies on habituation in patients with suicidal behavior. Participants were divided in two groups: Suicide Attempters (SA) and Controls without suicidal behavior (Ctrl). The task consisted of 32 trials with repeated auditory stimuli at 0.5 Hz delivered to both ears through headphones. Bioelectric signals were recorded by conventional EEG (10-20

system) to measure three P200 parameters: rate of rise (RR), amplitude (Amp) and peak latency (Lat), which were each analyzed separately by groups. The degree of habituation was explored by normalizing to 100% the first wave and calculating the % of decrease of the second wave for each P200 parameter on each group. 59 subjects participated, 29 (49.2%) of SA and 30 (50.8%) Ctrl, with no difference in age ($p = 0.693$). As expected, the RR was higher in SA (36.4 ± 15.7 μV/ms) vs Cntrl (35.2 ± 16.2 μV/ms). Likewise, the Amp was lower in SA (3.9 ± 1.7 μV) vs Cntrl (4.1 ± 1.9 μV). However, these differences were not significant. Instead, Lat showed differences ($p=0.038$, 1-tail), being SA faster (221.0 ± 37.4 ms) than Ctrl (228.5 ± 17.3 ms). The habituation analyses showed a reduction to 99.3% in the RR of SA and 90.6% in Ctrl. The Amp in SA was 95.7% and 91.7% in Ctrl. Finally, the Lat was 92.5% and 94.7% in SA and Ctrl, respectively. According to the hypothesis, the SA showed a clear tendency to smaller reduction in RR and Amp of the second wave, indicating lower habituation. The effects are consistent with low serum serotonin levels in SA and lower cortical performance than patients without history of suicide attempts. These results open the possibility of using P200 parameters as neurobiological markers in vulnerable subjects and reinforce timely preventive measures.

Disclosures: O.H. Hernandez: None. **F.I. Santos-gallegos:** None.

Poster

249. Human Social Cognition: Behavior, Mechanisms, and Disorders II

Location: Hall A

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Program #/Poster #: 249.11/AA14

Topic: H.02. Human Cognition and Behavior

Support: NIMH Grant MH077908
NIMH Grant MH102310
AAUW Career Development Grant

Title: Familial and lifetime history of depression: Alterations in the neural circuitry underlying reward and social cognition

Authors: *L. J. TEPFER[1], L. B. ALLOY[2], D. V. SMITH[1];
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Abstract: Major Depressive Disorder (MDD) is associated with alterations in reward processing and social cognition. Yet, it remains unclear whether a family history of MDD alone is associated with these changes, despite the absence of a personal experience with depression. To address this question, we analyzed task-based fMRI data of 279 participants (males, 120; 159 females; age, 22-36; mean ±SD, 28.45 ±3.75 years) from the Human Connectome Project (HCP). We subdivided participants into three groups: 71 with lifetime history of MDD (DEP), 103 with a family history of MDD (FAM) and 105 healthy controls (HC). All participants completed the

HCP's social and reward processing tasks. These tasks evoked increased activation in expected regions, including the ventral striatum and ventromedial prefrontal cortex (vmPFC) during reward processing, and temporoparietal junction and vmPFC for the social task. We also found group differences in activation, where DEP demonstrated increased lingual gyrus activity relative to FAM during the reward task. Next, to assess group differences further, we used the vmPFC region that was active in both tasks as a seed region in PPI analysis. This analysis revealed two key results. First, FAM showed increased vmPFC functional connectivity with the orbitofrontal cortex (OFC) during social cognition relative to the DEP group. Second, the FAM group also had increased vmPFC-anterior cingulate gyrus connectivity relative to HCs during the social task. Together, these data suggest that the presence of neural alterations in the absence of a personal history of MDD implies that parental MDD may be sufficient to initiate neural vulnerabilities to future depressive episodes.

Disclosures: L.J. Tepfer: None. **L.B. Alloy:** None. **D.V. Smith:** None.

Poster

249. Human Social Cognition: Behavior, Mechanisms, and Disorders II

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Topic: H.02. Human Cognition and Behavior

Support: Eunice Kennedy Shriver National Institute of Child Health and Human Development contract #HHSN275201000007C
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NIH/ NICHD IR21HD073549- 01A1
NIH Director's New Innovator Award #1DP2DA03515601

Title: Is socioeconomic status associated with different neural pathways to cognitive control?

Authors: *C. N. CASCIO[1], M. J. FARAH[2], G. M. LAWSON[3], E. B. FALK[3];
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Abstract: Response inhibition is a critical predictor of many important outcomes in life, ranging from educational attainment to health behaviors and outcomes. In parallel, socioeconomic status (SES) predicts several of these important outcomes, as well as a variety of cognitive abilities. The current study extends our understanding of SES and cognition by examining brain activity associated with response inhibition, during the key developmental period of adolescence. Adolescent males, aged 16-17 (*N*=81) completed a response inhibition (go/no-go) task while undergoing fMRI and reported on their SES following the scanning session. A region of interest (ROI) analysis showed that SES was associated with activation differences in the classic

response inhibition network, including the right inferior frontal gyrus, subthalamic nucleus, and basal ganglia (β=.35, t(68)=3.10, p=.003, r^2=.12, CI=[.13, .58]), despite the absence of an SES effect observed behaviorally (p>.05). A whole brain analysis revealed effects of SES as well as inhibitory performance in regions outside the classic response inhibition network including the angular gyrus and middle temporal gyrus (K>57, p=.001, corresponding to p<.05, corrected), which were in turn marginally associated with performance differences (β=-.20, t(68)=-1.71, r^2=.04, p=.091, CI=[-.24, .02]; β=-.20, t(68)=-1.65, r^2=.04, p=.104, CI=[-.17, .02]; respectively). These findings suggest that neural regions most widely implicated in successful inhibitory control in the published literature may be particularly relevant in samples of individuals who are high in SES.

Disclosures: **C.N. Cascio:** None. **M.J. Farah:** None. **G.M. Lawson:** None. **E.B. Falk:** None.

Poster

249. Human Social Cognition: Behavior, Mechanisms, and Disorders II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 249.13/AA16

Topic: H.02. Human Cognition and Behavior

Title: Transcranial alternating current stimulation aimed at the inferior frontal gyrus affects face perception and working memory but not self perception in healthy humans

Authors: T. A. BLESS[1], N. M. R. GOODMAN[2], P. MULVANY[1], J. L. CRAMER[1], *J. HONG[1], B. HARO[1], S. SCHUGAR[1], F. SINGH[1], **J. A. PINEDA**[1];
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Abstract: We tested whether non-invasive transcranial alternating current stimulation (tACS) aimed at the inferior frontal gyrus (IFG) affects self-perception, the semantic processing of faces, and/or cognition (namely working memory or WM). The IFG contains mirror neurons presumed to be involved in all these functions. By targeting the 8-12 Hz EEG mu rhythm, an indirect measure of mirroring and IFG activity, tACS was hypothesized to improve the processing of social functions through its entrainment of the mu rhythm. We studied this by examining subjects' self-drawing, recognition of human faces, and performance on a verbal WM task before and after tACS. A total of 80 subjects with no psychiatric diagnosis were randomly assigned to one of two studies (FACE or WM). Within each study, participants were randomly assigned to one of two conditions (STIM or SHAM) and underwent single-session testing. Each subject's EEG was recorded using a 20-channel dry EEG headset (Cognionics) during a baseline condition. In the FACE study, participants completed a self-perception task (Draw a Person: DAP-IQ) and an animate/inanimate face identification task. In the WM study, they were exposed to a 2-back verbal memory test. Stimulation consisted of 1mA, 20 min, 10 Hz, Anode - F3 (IFG),

Cathode - F8. In the FACE study, post-STIM compared to pre-STIM resulted in increased threshold of human face detection with participants showing a more difficult time determining what was a human face following stimulation. In contrast, post-STIM did **not** produce more detailed responses or increased performance on the self-perception test. There was also suppression in mu rhythm power over the left hemisphere during the self-perception task following tACS stimulation, but an inhibition of suppression over the right parieto-occipital hemisphere. In the WM study, post-STIM compared to pre-STIM resulted in increased accuracy and longer response times. No such effects were observed during the SHAM condition. Overall, results show that tACS stimulation of the IFG has effects on semantic processing of faces to make categorical judgments about animacy and on WM performance, but no effects on self-perception. Further analyses are ongoing to rule out the effects of task practice and of different groups.

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Poster

249. Human Social Cognition: Behavior, Mechanisms, and Disorders II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 249.14/AA17

Topic: H.02. Human Cognition and Behavior

Title: Impulsivity and risk behaviors correlated in adolescence

Authors: *A. R. WILLHELM[1], A. S. PEREIRA[2], R. M. DE ALMEIDA[3];
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Abstract: The neuromaturational trajectory of inhibition systems occurs throughout adolescence, which allows flexibility in the development of these systems. The willingness of adolescents to risk is due to maturational imbalance between a brain network involved in deliberative behavior, planned and directed to a goal and that is involved in affective processes. Shortly after puberty, the affective processing system undergoes rapid development, producing a greater sensitivity to reward that decreases in late adolescence. This research had 2 studies on impulsivity, aggression and alcohol use, the aim of this research was to discuss these aspects in groups of adolescents. The first study aimed to compare impulsivity, aggressiveness and alcohol in 115 male adolescents aged 14 to 17 years old. The study has 4 groups: juvenile offenders, students in regular school, athletes in a soccer team and students at a military school. The instruments used: questionnaire about substance use, the Barratt impulsiveness scale (BIS) and the State-Trait Anger Expression Inventory (STAXI). The results indicated that juvenile offenders had higher levels of anger feelings and impulsivity compared with other groups. Although the groups did

not differ in terms of alcohol experimentation, those who had already consumed alcohol presented higher scores on impulsivity and aggressiveness. Intense sports practice was associated with lower level of anger and studying in a military school with lower motor impulsivity. The second study aimed verify if adolescents with different levels of impulsivity (low, medium and high) also present different levels of aggressiveness and inhibitory control. The sample of this study consisted of 285 healthy pre-adolescents and adolescents aged 10 to 18 years old. The instruments used were: BIS, STAXI, Go No Go Task, Strengths and Difficulties Questionnaire. The results suggest that high levels of impulsivity are related to lower inhibitory control, greater aggressiveness, greater problems related to mental health and greater use of substances. Adolescents with lower levels of impulsivity have greater inhibitory control and less risk behaviors such as alcohol and drug use. It was possible to observe from the results of these two studies that alcohol consumption is present in all contexts, with no differences between them. It was also possible to observe a relationship between impulsiveness and aggressiveness, in which more aggressive adolescents are also more impulsive and end up putting themselves in risk situations. It has not yet been possible to perform biological material analyzes, this will be done the next steps (cortisol analysis and genetic analysis).

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Poster

249. Human Social Cognition: Behavior, Mechanisms, and Disorders II

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Topic: H.02. Human Cognition and Behavior

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Title: Specific neural correlates integrate flow and social experience

Authors: *M. H. SHEHATA[1,2], M. CHENG[1,3,4], D. WU[1], C.-H. TSENG[5], S. NAKAUCHI[2], S. SHIMOJO[1];
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Abstract: Team flow occurs when a group of people falling into the zone together while contributing to complete a particular task. For example, it occurs in sports teams, music ensembles, dance squads, or a group video game playing. Many studies suggest team flow as a qualitatively different experience from the simple aggregation of individual flow and regular

social interaction. These suggestions are based only on behavioral observations including enhanced creativity, performance, or feelings. However, whether team flow is a neurally distinguishable brain state is still unclear. Moreover, the neural correlates of individual flow and social interaction reported in the literature show conflicting neural activities at certain brain areas, such as the prefrontal cortex, raising the question: what happens to these brain areas when these two experiences are integrated?Here, we addressed these questions using a music rhythm video game played in a social context and electroencephalogram hyper-scanning. We manipulated flow through scrambling the music and manipulated social interaction through hiding the partner's body and feedback; effective manipulations were confirmed using psychometric ratings. Moreover, we used the inhibition of the auditory-evoked potential to a task-irrelevant stimulus as an objective measure of the subjective flow experience. Topological data analysis showed higher beta/gamma frequency power at the left temporal lobe specific to team flow condition that the individual flow and non-flow conditions. This power effect was localized to the superolateral, middle, and inferior temporal brain regions, known for their role in multimodal integration. To understand how the brain combines the flow and social experiences, we used unsupervised machine learning clustering of the beta/gamma power signals to detect the neural correlates specific to each experience during team flow. This analysis revealed flow-specific clusters localized to the anterior frontal and cingulate cortex, social-specific clusters localized to the medial and inferior frontal cortex, and team flow-specific clusters localized to the temporal and parietal cortex. Our data indicate that team flow is a qualitatively unique brain state, not a mere quantitative summation of the social and individual flow experience, where temporal lobe regions are possibly recruited to integrate component experiences. Also, our data show that the brain resolves the combination of experiences with opposing neural activities through creating subdivisions each showing neural signals for one experience.

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Poster

249. Human Social Cognition: Behavior, Mechanisms, and Disorders II

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Program #/Poster #: 249.16/AA19

Topic: H.02. Human Cognition and Behavior

Support: NIMH K23 MH074895

Title: Dorsal anterior cingulate cortex activation linked with executive and attentional functioning in bipolar disorder

Authors: *K. SRETAVAN WONG, T. CHOU, A. TESTO, K. K. ELLARD, D. DOUGHERTY, T. DECKERSBACH;
Massachusetts Gen. Hosp., Boston, MA

Abstract: The Frontal Systems Behavior Scale (FrSBe; Grace, Stout, & Malloy, 1999) and the Adult ADHD Self-Report Scale (ASRS; Kessler et al., 2005) are self-report questionnaires, which assess behavioral deficits associated with executive functioning and attentional difficulties. On a superficial level, these measures may seem to tap into different constructs. However, specific subscales of these questionnaires have a conceptual overlap, in particular, FrSBe's disinhibition and executive dysfunction subscales and ASRS' inattention and hyperactivity subscales. Since both scales assess deficits commonly observed in Bipolar Disorder (BD), we explored the relationship between FrSBe and ASRS and investigated the neural correlates of these measures in individuals with Bipolar Disorder and healthy controls. The sample consisted of 62 individuals (n=31 individuals with BD and n=31 healthy controls [HC]). All subjects completed the FrSBe, ASRS, as well as the Multi-Source Interference Task (MSIT; Bush & Shin, 2006) during fMRI scanning with a Siemens Trio 3T MRI scanner. The MSIT assesses the ability to deal with interfering, task-irrelevant stimuli. We extracted beta weight values from the dorsal anterior cingulate cortex (dACC; MNI coordinates = -6, 26, 30) for each subject using SPM MarsBaR, then conducted a Pearson's *r* correlational analyses. Total FrSBe scores were positively correlated with total ASRS scores (r=0.68, $p\leq$0.001; BP group: r=0.59, $p\leq$0.001, HC group: r=0.56, $p\leq$0.001). Both FrSBe and ASRS scores were correlated with dACC activation. Specifically, FrSBE scores were negatively correlated with dACC activation (r=-0.28, p=0.03; BD group: r=-0.19, p=0.31; HC group r=-0.06, p=0.75). Total ASRS scores were negatively correlated with dACC activation (r=-0.35, p=0.005; BD group: r=-0.39, p=0.03, HC group: r=-0.23, p=0.21). Taken together, these findings suggest that the two scales do not measure different constructs. dACC appears to be a common neural correlate for both scales. This finding may provide support for the dACC as a potential target for both frontal lobe behavior syndromes and ADHD symptoms in BD.

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Poster

249. Human Social Cognition: Behavior, Mechanisms, and Disorders II

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Program #/Poster #: 249.17/AA20

Topic: H.02. Human Cognition and Behavior

Title: Political moralization enhances ERPs to social information and decreases social conformity

Authors: *K. J. YODER, J. DECETY;
Psychology, Univ. of Chicago, Chicago, IL

Abstract: American politics is increasing marked by political polarization and a belief that specific policy positions are morally right or wrong, rather than a matter of opinion. The current study examined the neural mechanisms which distinguish such moralized political views and identified factors which can predict social conformity. Participants first indicated their support or opposition to a set of specific socio-political issues, as well as the extent to which their views were related to their fundamental beliefs about right and wrong. At least one week later, they underwent high-density electroencephalography (hd-EEG) while viewing photographs of political protests and indicated their support for the protestors. Prior to watching each photo, participants read the issue that led to the protest, then saw the percentage of other people who supported or opposed that issue. Hierarchical linear models were used to regress in-person support ratings on moralization, social support, and prior support. As predicted, moralized views were less sensitive to social influence, and demonstrated counter-conformity when the majority of others disagreed with the participant's own views. Two sets of event-related potentials (ERPs) were generated by time-locking to the social support information or the protest photo. Greater amplitudes for parietal P2 were found when social consensus was congruent with participants' own political views, and this effect was stronger for moralized views. This interaction also predicted subsequent ratings, with greater moral congruency ERP response predicting less social conformity. Photographs of protests regarding moralized issues compared to non-moralized issues elicited enhanced frontal P1 amplitudes and later positive potentials. Taken together, these findings indicate that moralized political views are less susceptible to social conformity because moralization alters early representations of social information.

Disclosures: K.J. Yoder: None. **J. Decety:** None.

Poster

249. Human Social Cognition: Behavior, Mechanisms, and Disorders II

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Program #/Poster #: 249.18/AA21

Topic: H.02. Human Cognition and Behavior

Support: NIH P50 MH0942581
NIH U01 NS103780
Kiwanis International Neuroscience Research Foundation

Title: Patients with damage to the amygdala report increased authoritarianism and religious fundamentalism

Authors: *E. W. ASP[1], L. KHAN[2], D. TRANEL[3];
[1]Neurol., Univ. of Iowa, Iowa City, IA; [2]Psychology, Hamline Univ., Saint Paul, MN; [3]Dept Neurol, Univ. Iowa, Iowa City, IA

Abstract: Recent studies have established that patients with damage to putative emotion-related brain structures (e.g., ventromedial prefrontal cortex lesions; vmPFC) can show a pattern of high authoritarianism, the tendency to submit to authority and assail others in the name of authority, and high religious fundamentalism, the tendency to hold religious beliefs with an immutable, unjustified certainty. Research has shown that both patients with vmPFC damage and those with amygdala damage have increased credulity to explicitly-labeled false information. To account for these lesion patients' tendency toward belief acceptance, we have developed the False Tagging Theory (FTT), which proposes that the ventromedial prefrontal cortex and the amygdala are critical for somatic "false tags" in the psychological concept of doubt. If lesion-induced-credulity is a causal factor for increased authoritarianism and religious fundamentalism, then patients with amygdala damage should show increased authoritarianism and religious fundamentalism. In a confirmatory study, we show that patients with damage to the amygdala report an abnormally high pattern of authoritarianism and religious fundamentalism relative to healthy adults and brain damaged comparison patients. The findings support the FTT and suggest that the amygdala is critical for psychological doubt and resistance to authoritarian persuasion.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIMH Conte Center Grant 2P50MH094258

Title: Single-neuron, sEEG and fMRI recordings in human epilepsy patients during movie watching: An alternate window on neural selectivity

Authors: *J. DUBOIS[1], U. KELES[3], J. M. TYSZKA[3], C. M. REED[1], J. M. CHUNG[1], R. ADOLPHS[3], A. N. MAMELAK[1], U. RUTISHAUSER[2];
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Abstract: Neuroscientists are increasingly using naturalistic stimuli, such as movies - these engaging stimuli increase patient compliance, better match ecological conditions and sample a richer array of features than typical static, trial-based experiments. It has been shown that movie stimuli evoke neural activity that is reproducible across experimental sessions within subject, in

primary sensory and some higher-level brain areas. This observation was recently leveraged to cluster macaque AF face patch cells based on their correlation with whole-brain fMRI activity recorded in a different session (while macaques watched the same movie). Here we present a dataset which we collected over the past three years to study neural responses while subjects watch an 8-min TV episode (a shortened version of Alfred Hitchcock's Bang You're Dead, from 1961). The dataset features epileptic patients (N=10) being evaluated for seizure focus localization with sEEG depth electrodes (hybrid macro-micro electrodes). Before electrode implantation, the patients are scanned while watching the audiovisual movie twice in a 3T MRI scanner, using state-of-the-art multiband EPI acquisition on a 32-channel head-receive coil (2.5mm isotropic voxels, TR=1000ms). Then, after implantation of intracranial electrodes, the patients watch the same movie again, twice. Electrode placement was entirely guided by clinical presentation; most patients were implanted with 5 hybrid electrodes bilaterally, targeting the following medial regions: orbitofrontal cortex (OFC), anterior cingulate cortex (ACC), pre-supplementary motor area (SMA), hippocampus (HIP) and amygdala (AMY). Additional electrodes were implanted on a case-by-case basis. In total across 10 subjects, we collected 18 unique sessions, recorded from 928 sEEG contacts and 848 microwires (from which we isolated 120 putative single units in HIP, 180 in AMY, 294 in ACC, 159 in SMA, and 154 in OFC). We found high test-retest reproducibility of movie-evoked activity in the electrophysiological data and in the fMRI data at a subset of the locations sampled by electrodes. We also found fMRI and electrophysiological responses to different features of the movie, such as event boundaries in HIP, or faces in AMY. These responses were generally concordant between electrophysiology (sEEG) and fMRI as expected, with a positive relationship between evoked gamma power and evoked BOLD, and a negative relationship between evoked theta power and evoked BOLD. We present insights from single-unit, sEEG and BOLD fMRI selectivity in naturalistic conditions, and their relationship.

Disclosures: **J. Dubois:** None. **U. Keles:** None. **J.M. Tyszka:** None. **C.M. Reed:** None. **J.M. Chung:** None. **R. Adolphs:** None. **A.N. Mamelak:** None. **U. Rutishauser:** None.

Poster

249. Human Social Cognition: Behavior, Mechanisms, and Disorders II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 249.20/AA23

Topic: H.02. Human Cognition and Behavior

Support: Brain behavior research Foundation.

Title: Effects of social cognitive training on the neural system of theory of mind in healthy adults

Authors: *V. G. PATRON ROMERO[1], C. I. HOOKER[2], K. M. HAUT[3], D. DODELL-FEDER[4], E. GUTY[5], M. NAHUM[6];
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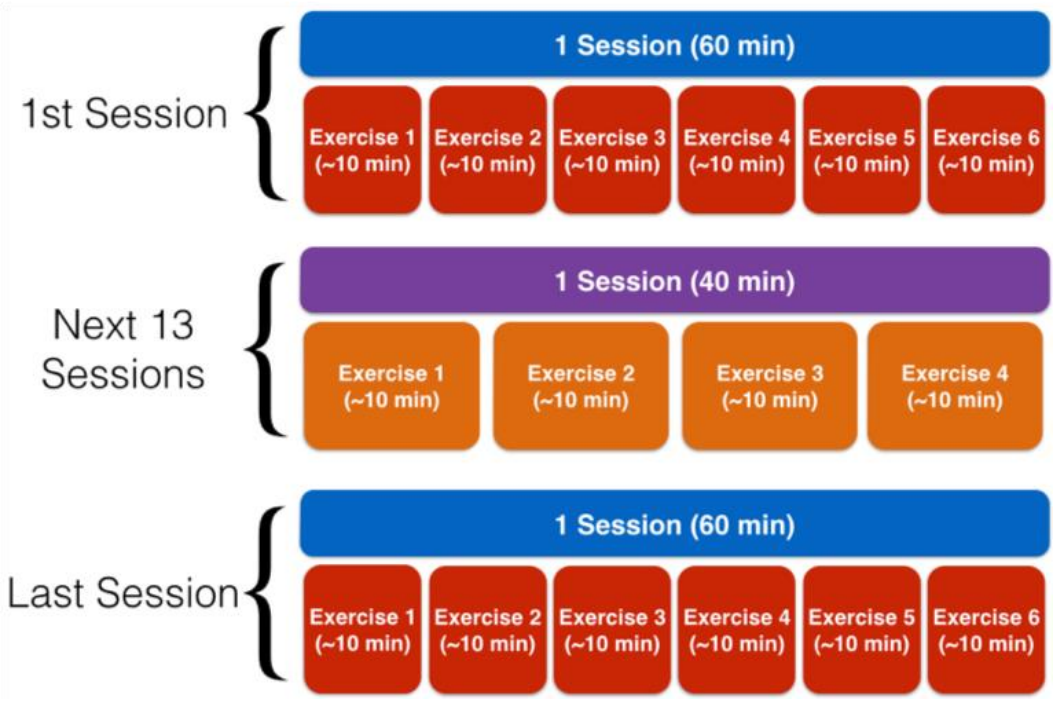
Abstract: Theory of Mind (ToM), the ability to infer another person's mental state, is essential for social functioning. ToM and mental state reasoning neural networks include medial prefrontal cortex, precuneus and temporoparietal junction. Dysfunction of ToM and associated networks has been demonstrated in schizophrenia, autism, and other disorders making it a target for cognitive remediation. However, most cognitive remediation programs focus on cognition and little is known about neural effects of social cognition training in healthy adults. The current study examined neural changes in the ToM network after social cognition training. Fifty-one healthy adults completed an fMRI ToM task before and after a 10.5-hour computerized intervention of social cognition exercises (N=24) or 'placebo' computer games (N=27). While undergoing fMRI participants read stories describing another person's thoughts, emotions or appearance followed by a statement about a subsequent action or behavior by that person. Subjects were asked to judge whether the behavior was coherent in the context of the story. Stories were design to match complexity. Contrast of (Thought + Emotion) -Appearance was compared FSL neuroimaging software. When compared to placebo intervention, participants who completed social cognitive training demonstrated an increase in activation in the ToM network, as well as cognitive control regions. Social cognitive training induced changes in activation in areas associated with theory of mind and cognition in healthy individuals.



Disclosures: **V.G. Patron Romero:** None. **C.I. Hooker:** A. Employment/Salary (full or part-time):; Rush University Medical Center. **K.M. Haut:** A. Employment/Salary (full or part-time):; Rush University Medical Center. **D. Dodell-Feder:** A. Employment/Salary (full or part-time):; University of Rochester. **E. Guty:** None. **M. Nahum:** A. Employment/Salary (full or part-time):; The Hebrew University of Jerusalem.

Poster

249. Human Social Cognition: Behavior, Mechanisms, and Disorders II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 249.21/AA24

Topic: H.02. Human Cognition and Behavior

Title: The effect of a brain training session on attention and consistency of optimal response on implicit bias

Authors: A. N. CAYCE, W. O. NEESE, P. EBO, K. A. MAGAÑA, ***J. P. ABARA**; California State Univ. Northridge, Northridge, CA

Abstract: Task efficiency recruits attentional components that affect output delivery such as accuracy of response and response time. Scharnowski, Hutton, Josepj, Weiskopf, and Rees (2012) and Sinotte and Coelho (2007) documented that brain training with the use of neurofeedback enhances perceptual processing, performance in working memory and attention task. Mozanezhad, Jeddi, & Nazari (2013) have particularly highlighted the favorable effect of neurofeedback as a training tool for focus, concentration, and calmness leading to optimal accuracy and reaction time. The aim of this experiment is to evaluate the consistency of optimal performance during an attention and implicit bias task after a neurofeedback session. A continuous performance task was used before and after neurofeedback training. Evaluation of performance on an attention task across participants before and after neurofeedback yielded significant results. Reaction time after a neurofeedback session was faster than the reaction time before a neurofeedback session, $F(1, 15) = 4.56$, $p = .049$. This finding provides support for the use of neurofeedback procedure for optimal performance such as during an implicit bias task. The effect of neurofeedback on possible reduction of implicit bias performance is discussed.

Disclosures: **A.N. Cayce:** None. **W.O. Neese:** None. **P. Ebo:** None. **K.A. Magaña:** None. **J.P. Abara:** None.

Poster

249. Human Social Cognition: Behavior, Mechanisms, and Disorders II

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 249.22/AA25

Topic: H.02. Human Cognition and Behavior

Support: HSE Basic Research Program
Russian Academic Excellence Project '5-100'
Russian Scientific Foundation grant No. 16-15-00300

Title: An association of subjective experience on planning and visual thoughts with resting-state EEG dynamic

Authors: G. PORTNOVA[1,2], K. LIAUKOVICH[1], ***V. MOISEEVA**[3], O. MARTYNOVA[4,3]; [1]Inst. of Higher Nervous Activity and Neurophysiol. of RAS, Moscow, Russian Federation; [2]The Pushkin State Russian Language Inst., Moscow, Russian Federation; [3]Higher Sch. of Econ., Moscow, Russian Federation; [4]Inst. of Higher Nervous Activity and Neurophysiol. of RA, Moscow, Russian Federation

Abstract: One of the approaches to gain knowledge on the neural underpinnings of consciousness is finding an association between electrical brain activity (EEG) and subjective experience during resting state. Amsterdam Resting-State Questionnaire (ARSQ) is based on retrospective self-report and designed to assess such experience during mind wandering qualitatively. We tested a correlation of rating on ARSQ with spectral and dynamic parameters of EEG recorded in 49 healthy volunteers during the 10-min resting session. The participants filled ARSQ immediately after the rest. We investigated both linear (1 Hz-band power spectral density - PSD) and dynamic features (standard deviation and frequency of Hilbert envelope) of EEG averaged for the whole resting-state segment. Besides, we conducted a procedure of k-mean clustering based on PSD, localization of components retrieved by independent component analysis for 10-sec EEG epochs to assess spectral and temporal variability of EEG. A correlation analysis showed that the increase of PSD and cluster duration of the high-frequency alpha rhythm (12-13 Hz) in central and frontal areas was positively associated with the rating of experienced thoughts related to Planning. Previous studies also reported in the association of the upper alpha band 12-13 Hz in frontal areas with direct attention, planning of motor tasks and social interactions (Naeem et al., 2012). Our findings are partially consistent with data of Diaz et al., 2016 reported that higher alpha/theta ratio was correlated positively with Planning. Importantly, our results indicate that not wide-band alpha but the upper alpha band of 12-13 Hz, its power and contribution in the resting-state EEG with specific localization in the frontal and central areas, predicts higher ratings of Planning in ARSQ. On the contrary, a contribution of the cluster with low PSD of delta band 2-3 Hz in the frontal areas showed a negative association

with Planning. The participants with higher ARSQ scores of Visual Thoughts had a higher standard deviation of the wideband (1-30 Hz) Hilbert envelope. The higher standard deviation of envelope frequency may indicate higher variability in EEG dynamic increasing with the degree of the cognitive activity (Natarajan et al., 2004). Overall, our data suggest that the dynamic properties of EEG reflect cognitive states assessed by ARSQ.

Disclosures: V. Moiseeva: None. **G. Portnova:** None. **K. Liaukovich:** None. **O. Martynova:** None.

Poster

250. Transcriptomic and Genomic Analyses

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 250.01/AA26

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Title: Tumour necrosis factor-alpha (TNF-α) gene polymorphisms and risk of intracerebral hemorrhage in North Indian population: A hospital based study

Authors: *R. SAGAR[1], A. KUMAR[1], A. K. YADAV[1], D. PATHAK[2], A. MISHRA[1], D. RAWAT[1], D. DASH[1], A. K. SRIVASTAVA[1], S. VIVEKANANDHAN[3], G. GUPTA[4], K. PRASAD[1];
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Abstract: Background: TNF-α gene is an important proinflammatory cytokines and thought to be involved in pathogenesis of ICH with damaging effects on cerebral arteries by promoting inflammation and apoptosis in vascular cells.
Aim & Objective: To investigate the relationship between *TNF-α (C857T, T1031C, G488A, G308A)* gene polymorphisms and risk of ICH in North Indian population.
Methods: In this present case-control study, DNA isolated by chloroform-phenol method and genotyping was performed by MALDI-TOF MassARRAY method for 250 patients and 250 age-sex matched controls. Frequency distribution of genotypes and alleles were compared between cases and controls by using conditional logistic regression.
Results: Mean age of patients and controls were 54.9±12.8 and 55.5±12.8; 35.2% were female. Frequency distribution of alleles was consistent with HWE. Conditional logistic regression analysis showed a significant association between *TNF-α (T1031C)* and risk of ICH under recessive model (OR=2.13; 95%CI 1.15 to 3.94; p=0.02) and after adjusting co-variates (OR=2.22; 95%CI 1.02-4.83; p=0.05) but not in under dominant model. We did not observe the significant relationship between *TNF-α (C857T, G488A, G308A)* and risk of ICH under recessive and dominant model. We did not observe the significant association between TNF-α *(C857T,*

T1031C, G488A, G308A) gene polymorphisms and risk of ICH under allelic model.
Conclusion: *TNF-α (T1031C)* gene polymorphism significantly associated with increased risk of ICH under recessive model in North Indians.

Disclosures: R. Sagar: None. **A. Kumar:** None. **A.K. Yadav:** None. **D. Pathak:** None. **A. Mishra:** None. **D. Rawat:** None. **D. Dash:** None. **A.K. Srivastava:** None. **S. Vivekanandhan:** None. **G. Gupta:** None. **K. Prasad:** None.

Poster

250. Transcriptomic and Genomic Analyses

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 250.02/AA27

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: HRB and Epilepsy Ireland Grant

Title: Comparison of human plasma microRNA expression analysis techniques in paediatric epilepsy patients: RNASeq versus nanostring

Authors: *N. ENRIGHT[1,2], G. BRENNAN[1], N. NGYUEN[1], M. D. KING[2], D. HENSHALL[1]; [1]Physiol. and Med. Physics, Royal Col. of Surgeons In Ireland, Dublin, Ireland; [2]Neurol., Childrens Univ. Hosp. Temple Street, Dublin, Ireland

Abstract: MicroRNAs (miRNA) are small non-coding RNAs approximately 22 nucleotides long which regulate protein expression through translational inhibition or, less often, mRNA degradation. MiRNAs have been linked to epilepsy in multiple ways. Many are enriched in brain tissue but expressed only at low levels in other tissues. Their presence in plasma could indicate neuronal injury or controlled release via exosomes. Multiple studies have been undertaken attempting to identify possible miRNA biomarkers of epilepsy. Plasma or serum are commonly used biofluids due to the relative ease of obtaining them, and their stability for prolonged periods once frozen. To date studies have used, mainly, a combination of RNA sequencing and RT-qPCR to identify possible biomarkers in plasma. A newer technique, Nanostring, has been used more often recently. Nanostring uses direct quantification to analyse up to 800 miRNA targets. To date very few articles have looked at expression analysis across these platforms, especially in plasma miRNA expression.
This study aimed to compare miRNA differential expression analysis from the plasma of paediatric epilepsy patients and controls in order to identify possible biomarkers. We used the Illumina miSeq technology for RNA sequencing. RNA from the same plasma samples was then run on the Nanostring platform. Differentially expressed miRNAs from both platforms were then validated using RT-qPCR. Results show that differential expression data from RNA sequencing is comparable with Nanostring data. Several differentially expressed miRNAs from both

platforms were validated using RT-qPCR, confirming these results. The most commonly expressed miRNAs in plasma were also conserved across platforms. This study suggests that Nanostring could be used as an initial screening tool to identify possible differentially expressed miRNAs in biofluids; as well as being used to validate large number of differentially expressed miRNAs identified through RNASeq. Using these techniques we have successfully identified possible plasma biomarkers of paediatric epilepsy.

Disclosures: **N. Enright:** None. **G. Brennan:** None. **N. Ngyuen:** None. **M.D. King:** None. **D. Henshall:** None.

Poster

250. Transcriptomic and Genomic Analyses

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 250.03/AA28

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: CHDI Foundation

Title: Characterization of the gene regulatory network of HTT

Authors: *J. COCHRAN, B. S. ROBERTS, M. MACKIEWICZ, E. PARTRIDGE, B. A. MOYERS, A. A. HARDIGAN, K. R. DAY, K. NEWBERRY, D. E. MOORE, R. M. MYERS; Hudsonalpha Inst. For Biotech., Huntsville, AL

Abstract: A gene regulatory network comprises all of the cis-acting regulatory elements (CREs) such as enhancers and promoters, and the DNA binding proteins, transcription factors (TFs) and others, which in concert control the expression of a given gene. We have sought to thoroughly characterize the gene regulatory network of *HTT* for several reasons. An understanding of the regulation of *HTT* expression would facilitate not only the interpretation of the results in the field to lower *HTT* expression through various means, but also the direction of future efforts. Furthermore, by establishing the TFs responsible for the regulation of *HTT* expression, we hope to find clues to its normal function. To identify *HTT* CREs, we have employed a myriad of genomic assays in human cultured neurons derived from induced pluripotent stem cells as well as in several commonly used cell lines. Among these are ATAC-seq to measure open chromatin, ChIP-seq to measure TF occupancy and histone post-translational modifications, Capture-C to find 3-dimensional chromatin interactions with the *HTT* promoter, and STARR-seq to assay enhancer activity of millions of DNA test elements. From the analysis of these data, we nominated several regions as enhancers of *HTT* and tested the effects of their loss-of-function on *HTT* expression through targeted dCas9-KRAB constructs, with robust validation of target engagement in positive controls in our hands. For several of the putative *HTT* CREs, ablation resulted in a significant decrease in *HTT* expression, further establishing their role in the *HTT*

gene regulatory network. From these newly identified *HTT* enhancers and the well-established promoter, we looked for known TF motifs within "TF footprints" revealed by ATAC-seq data. By comparing these motifs with existing ChIP-seq data (non-neuronal sources), and filtering for mRNA expression in cultured neurons, we identified a set of TFs potentially binding to *HTT* CREs and thus perhaps regulating *HTT* expression. By employing ChIP-seq targeting these TFs in cultured neurons, we confirmed occupancy at the *HTT* promoter and at a subset of CREs for several candidates.

Disclosures: J. Cochran: None. **B.S. Roberts:** None. **M. Mackiewicz:** None. **E. Partridge:** None. **B.A. Moyers:** None. **A.A. Hardigan:** None. **K.R. Day:** None. **K. Newberry:** None. **D.E. Moore:** None. **R.M. Myers:** None.

Poster

250. Transcriptomic and Genomic Analyses

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 250.04/AA29

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: NIMH Grant F30 MH1116654
NIMH Grant 1R01MH116999

Title: Toward an *in vivo* method for cell-type specific, multiplex assays of neuropsychiatric-trait associated transcriptional elements

Authors: *B. MULVEY[1], B. COHEN[1], J. D. DOUGHERTY[2];
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Abstract: The majority of co-inherited regions of single-nucleotide polymorphisms (SNPs) associated with neuropsychiatric disorders such as schizophrenia and major depressive disorder (MDD) are not protein coding genomic regions. Nonetheless, twin cohorts and SNP-based heritability both have repeatedly demonstrated heritability of these disorders. The consensus hypothesis regarding the role of these variants in complex traits, such as psychiatric disorders, is that the presence of one or more SNPs in a disease-associated locus results in altered activity of transcriptional regulatory elements, such as enhancers and promoters. While computational tools to infer regulatory SNPs have flourished in recent years, means of systematic screening and identification of functional regulatory SNPs have been put to limited use, mostly in non-neural cell types. Here, we present evidence that massively parallel reporter assays (MPRAs), which utilize unique RNA barcodes to ascertain the activity of their paired regulatory element, can be combined with *in vivo* cell-type specific transcriptomic tools such as translating ribosome affinity purification (TRAP) at modest scales. As the transcriptional regulatory milieu varies

substantially among cell types, and changes in gene expression in neuropsychiatric disorders suggest perturbations of cell subpopulations, this combinatorial method creates new avenues to identify—and subsequently dissect the consequences of—prioritized non-transcribed genomic loci associated with disease in the living brain, targeted to the cell types or brain regions in which the disease process manifests. Moreover, the ability to perform these assays *in vivo* enables study of variables in gene regulation which are not amenable to *in vitro* study, such as sex, development, and aging.

Disclosures: B. Mulvey: None. **B. Cohen:** None. **J.D. Dougherty:** None.

Poster

250. Transcriptomic and Genomic Analyses

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 250.05/AA30

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: NIMH Intramural Program

Title: Polygenic contributions to antipsychotic response in schizophrenia spectrum illness

Authors: *M. R. HAMBORG[1], D. P. EISENBERG[3], B. KOLACHANA[2], M. D. GREGORY[4], B. ZOLTICK[2], J. A. APUD[5], K. F. BERMAN[6];
[1]Clin. and Translational Neurosci. Br., [2]Natl. Inst. of Mental Hlth., Bethesda, MD; [3]Clin. and Translational Neurosci. Br., Natl. Inst. of Mental Hlth., Bethesda, MD; [4]NIMH/NIH, Bethesda, MD; [5]NIH, Natl. Inst. of Mental Hlth., Bethesda, MD; [6]Clin. and Translational Neurosci. Br., NIH/National Inst. of Mental Hlth., Bethesda, MD

Abstract: Background: Heterogeneity in treatment response to antipsychotic medication in schizophrenia is substantial, with many individuals responding partially or not at all. Hypothesizing that some of this heterogeneity is due to previously reported genetic factors, we evaluated the correspondence between response to antipsychotic medication during a blinded medication withdrawal protocol and polygenic antipsychotic response score (PARS).
Method: Inpatients with schizophrenia or schizoaffective disorder (N=49, 29 +/- 7.5 mean age, 12 females, all Caucasian) participated in a blinded medication withdrawal protocol involving an antipsychotic monotherapy arm and placebo-only arm, each lasting 4-6 weeks. Blinded raters assessed symptoms with the Positive and Negative Syndrome Scale (PANSS), and scores were calculated relative to the change between average of symptoms on and off medication. PARS were generated based on results from a previous genome-wide association study of antipsychotic medication response (McClay et al., 2011). First, weighted sums of predictive SNPs carried were created for each individual across multiple p-value thresholds. Next, using principal components analysis, the first principal component, best reflecting the most inclusive threshold scores, was

adopted as the score for analysis. Five scores were generated based on the five different medication groups originally studied in McClay et al. Finally, correspondence between PANSS total change and the PARS was conducted in R using a general linear model controlling for population stratification effects.
Results: A significant association between change in PANSS scores and the quetiapine PARS was found (p = .018 uncorrected) in the expected direction, such that patients who showed greater clinical improvement on active relative to placebo treatment were more likely to have cumulative genetic variation associated with response to treatment.
Conclusion: This study provides preliminary corroborative evidence for the hypothesis that heterogeneity in response to antipsychotic medication in schizophrenia may be in part due to underlying genetic differences. Our analyses revealed that genetic factors linked to response to quetiapine best predicted improved response, even though not all patients in this sample were treated with quetiapine, in agreement with the hypothesis that there may be common molecular mechanisms of action across non-clozapine atypical antipsychotic medications. Additionally, future studies with large, non-Caucasian samples are needed to build a more comprehensive understanding of molecular contributions to medication response variation.

Disclosures: M.R. Hamborg: None. **D.P. Eisenberg:** None. **B. Kolachana:** None. **M.D. Gregory:** None. **B. Zoltick:** None. **J.A. Apud:** None. **K.F. Berman:** None.

Poster

250. Transcriptomic and Genomic Analyses

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 250.06/AA31

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Title: Integrative DNA methylation and gene expression analysis of prefrontal cortex samples from Mexicans suicides

Authors: *A. L. ROMERO PIMENTEL, Sr[1], S. MONTERO-MUÑOZ[2], R. MENDOZA-MORALES[3], M. MORALES[2];
[1]Inst. Nacional De Medicina Genomica, Mexico City, Mexico; [2]Inst. Nacional de Medicina Genomica, Mexico City, Mexico; [3]Inst. de Ciencias Forenses, Mexico City, Mexico

Abstract: .

Disclosures: A.L. Romero Pimentel: None. **S. Montero-Muñoz:** None. **R. Mendoza-Morales:** None. **M. Morales:** None.

Poster

250. Transcriptomic and Genomic Analyses

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 250.07/AA32

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: NIH NIDA DA018310
USDA NIFA ILLU-538-909
NSF IOS 1121273
China Scholarship Council, CSC NO. 201606140027

Title: Gene network alterations in the cerebellum and striatum of mice selectively bred for increased voluntary wheel running behavior

Authors: *P. ZHANG[1,2], J. S. RHODES[3,6], T. GARLAND, Jr.[7], S. D. PEREZ[6], B. R. SOUTHEY[2], S. L. RODRIGUEZ-ZAS[2,4,5];
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Abstract: Mice selectively bred for high voluntary wheel-running behavior are helpful models for uncovering the neurological basis of the increased motivation for physical activity and reward-dependent behaviors. The goal of this work is to advance the understanding of the molecular mechanisms associated with increased voluntary wheel-running behavior in a High Runner line (HR) relative to non-selected control lines (Co). Transcript, pathway and network profiles were measured in brain regions related to reward processing and locomotor control, the cerebellum (Ce) and the striatum (St), using RNA-seq. The activity line-by-region interaction effects were explored based upon the transcriptome profiles of 16 samples from 4 groups: CeHR, CeCo, StHR, StCo. Overall, 154 genes exhibited activity line-by-region interaction, including neuropeptide genes that annotated to reward dependent processes and genes implicated in motor coordination. The Spearman correlation of the log2 (line fold change) between two regions based on the 154 genes was -0.034. Biological processes altered in the HR lines in a region-dependent manner included neurogenesis, cerebellum morphogenesis, adult locomotor behavior, cellular response to peptide, and hormone stimulus. The comparison of gene networks between two brain regions highlighted genes differentially expressed between HR and Co lines in a region-specific manner, including distal-less homeobox 5 gene (Dlx5), transcription factor AP-2-delta (Tfap2d), vitamin D receptor (Vdr), sine oculis homeobox homolog 3 (Six3), and transcription regulator LIM/homeobox protein Lhx1 (Lhx1). Results from this study suggest complex gene expression

profiles that are brain-region specific and involved in mediating genetic differences in motivation for increased voluntary exercise.

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Poster

250. Transcriptomic and Genomic Analyses

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 250.08/AA33

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: NIH BRAIN Initiative grant U19MH114830 to HZ
Allen Institute for Brain Science

Title: Multiplatform transcriptomic analysis of the primary motor cortex in adult mice

Authors: *B. TASIC, Z. YAO, L. GRAYBUCK, T. NGUYEN, K. SMITH, C. VAN VELTHOVEN, N. DEE, D. BERTAGNOLLI, J. GOLDY, O. FONG, B. LEVI, S. SUNKIN, M. HAWRYLYCZ, H. ZENG;
Allen Inst. For Brain Sci., Seattle, WA

Abstract: BRAIN Initiative Cell Census Network (BICCN) will create the most comprehensive multimodal cell type atlases of the mouse brain to date. To define optimal profiling strategy for single-cell transcriptomic methods, BICCN launched a Mini-Atlas project to test and compare multiple single-cell RNA-sequencing platforms on cells isolated from the primary motor cortex. We have generated data using several single cell RNA-sequencing methods including Smart-seq v4 whole cells, Smart-seq v4 nulcei, 10x whole cells, 10x nuclei, and SplitSeq cells. The number of cells or nuclei profiled range from 6K to 130K. We show that the number of genes detected by these methods vary significantly, and consequently, so does the number of clusters for each dataset produced by the same clustering pipeline. Lower gene detection can be compensated for by higher number of cells/nuclei sequenced. While all the datasets provide very similar major type separation, the cell type resolution as well as cell type composition differ among platforms. Comprehensive evaluation that accounts for sequencing depth, number of cells, cost per cell and cell type resolution suggested a combination of approaches to be pursued to profile the entire mouse brain.

Disclosures: B. Tasic: None. **Z. Yao:** None. **L. Graybuck:** None. **T. Nguyen:** None. **K. Smith:** None. **C. van Velthoven:** None. **N. Dee:** None. **D. Bertagnolli:** None. **J. Goldy:** None. **O. Fong:** None. **B. Levi:** None. **S. Sunkin:** None. **M. Hawrylycz:** None. **H. Zeng:** None.

Poster

250. Transcriptomic and Genomic Analyses

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 250.09/AA34

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: NIH Grant U19 MH114831
CZI Collaborative Computational Tools for the Human Cell Atlas (grant to EAM)

Title: Predicting cell type-specific gene regulatory elements through integrated analysis of single cell transcriptomes and epigenomes in mouse primary motor cortex

Authors: *K. KOLODZIEJ[1], F. XIE[2], C. VUONG[4], W. I. DOYLE[3], E. ARMAND[3], J. NERY[5], R. CASTANON[5], J. LUCERO[5], C. LUO[5], S. PREISSL[7], Z. YAO[8], B. TASIC[8], H. ZENG[8], E. M. CALLAWAY[4], M. BEHRENS[6], B. REN[7], J. R. ECKER[5], E. A. MUKAMEL[3];
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Abstract: The diverse identities of cell types in the mammalian cortex are established and maintained, in part, through the epigenetic regulation of gene regulatory elements such as enhancers. Knowledge of gene regulatory elements can enable design of targeted tools for assaying and manipulating neural cell types, however, the genomic locations of most cell type-specific enhancers and their target genes remain unclear. Integrated analysis of single cell transcriptomes and epigenomes, including DNA methylation and chromatin accessibility data, could enable comprehensive identification of cell type specific enhancers and their target genes. We developed a computational method for integrated analysis, SingleCellFusion, and used it to produce a multimodal neuronal cell type taxonomy including ~50 cell types in the mouse primary motor cortex using single-cell datasets (snRNA-Seq, snmC-Seq, and snATAC-Seq) from the BRAIN Initiative Cell Census Network (BICCN). For each cell type, we estimate gene expression, open chromatin regions and DNA methylation profiles by pooling information from corresponding cells in complementary datasets. Using the epigenomic data, we identify over 100,000 putative cell type specific enhancers by overlapping snATAC peak regions and differentially methylated regions (DMRs). Combining the epigenomes and transcriptomes, we find a clear negative correlation between DNA methylation at putative enhancers and the expression of nearby genes within 100 kilobases. Using this negatively correlated pattern, we predict the target genes of putative enhancers. Chromatin accessibility at predicted enhancers has a strong negative correlation with non-CG methylation level at target gene bodies, which further validates the putative enhancer to gene linkage derived from CG methylation and gene

expression.
These results show evidence of coordinated, cell type-specific patterns of gene expression and epigenetic signatures of active enhancers in mouse motor cortical neurons. Understanding the link between the epigenetic landscape and gene expression is important for revealing the diversity of neuronal cell types within the brain, a key to unraveling the complexity of neuronal circuitry and function.

Disclosures: **K. Kolodziej:** None. **F. Xie:** None. **C. Vuong:** None. **W.I. Doyle:** None. **E. Armand:** None. **J. Nery:** None. **R. Castanon:** None. **J. Lucero:** None. **C. Luo:** None. **S. Preissl:** None. **Z. Yao:** None. **B. Tasic:** None. **H. Zeng:** None. **B. Ren:** None. **M. Behrens:** None. **J.R. Ecker:** None. **E.M. Callaway:** None. **E.A. Mukamel:** None.

Poster

250. Transcriptomic and Genomic Analyses

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 250.10/AA35

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: NIH Grant U19 MH114831
CZI Collaborative Computational Tools for the Human Cell Atlas (EAM)
Howard Hughes Medical Institute (JRE)

Title: Integrated analysis of human and mouse cortical cell type epigenomes and transcriptomes reveals conserved and divergent neural regulation

Authors: *F. XIE[1], C. LUO[3], W. I. DOYLE[2], E. J. ARMAND[2], J. NERY[3], R. CASTANON[3], J. LUCERO[4], M. BEHRENS[4], J. R. ECKER[3], E. A. MUKAMEL[2];
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Abstract: Comparative analysis of the molecular signatures of neuronal cell types in different mammalian species, including gene expression and epigenetic markers of cell identity, can help to establish the biological significance of conserved features. Studies based on single-cell transcriptomes and epigenomes (Hodge et al 2018, Luo et al 2017) have enabled objective molecular characterizations of cell types in the human and mouse brain. These data suggest that broad classes and some fine subtypes of cortical neurons are conserved through mammalian evolution. However, it remains unclear to what extent cortical neurons are homologous, and what epigenetic signatures are conserved or divergent between homologous cell types in mouse and human.
We developed a data integration method, SingleCellFusion, that uses bigraph imputation to link measurements of single cell epigenomes (DNA methylation, snmC-Seq) or transcriptomes

(sc/snRNA-Seq) between human and mouse. SingleCellFusion identifies best matching groups of cells across species by taking advantage of the robust correlation between epigenetic marks and gene expression at orthologous genes in homologous cell types across species. We applied our method to over 9,000 single-nucleus DNA methylomes from mouse and human frontal cortex, and obtained ~40 integrated clusters across species. As expected, all major cell types are well aligned between human and mouse, with homologous epigenetic signatures across species. However, some cell types are strongly enriched in one species relative to the other. For example, chandelier cells (Pvalb+, Bnc5b+) are ~10-fold more abundant in human compared to mouse. In addition, we found some genes with divergent patterns of DNA methylation within homologous cell types across species. Finally, SingleCellFusion was used to integrate epigenetic and transcriptomic information for cell types in both species, providing integrated information about gene expression and regulation.
Our data integration method provides a framework for validation of predicted cell types in mammalian brain through evolutionarily conserved patterns of gene expression and epigenetic signatures. Moreover, aligning homologous cell types across species in a joint space allows systematic investigation of conserved and divergent features.

Disclosures: **F. Xie:** None. **W.I. Doyle:** None. **E.J. Armand:** None. **J. Nery:** None. **R. Castanon:** None. **J. Lucero:** None. **C. Luo:** None. **M. Behrens:** None. **J.R. Ecker:** None. **E.A. Mukamel:** None.

Poster

250. Transcriptomic and Genomic Analyses

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 250.11/AA36

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: U19MH11483

Title: Towards a multimodal cell type atlas of neurons in primary motor cortex of adult mice using Patch-seq

Authors: *F. SCALA[1], M. BERNABUCCI[1], D. KOBAK[2], J. R. CASTRO, JR[1], Y. BERNAERTS[2], S. LATURNUS[2], L. HARTMANIS[3], C. R. CADWELL[4], E. MIRANDA[1], D. RAMSKÖLD[3], Z. YAO[5], O. PENN[5], K. R. TOLIAS[1], B. TASIC[7], R. SANDBERG[3], X. JIANG[1], H. ZENG[6], P. BERENS[2], A. S. TOLIAS[1];
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Abstract: Neurons exhibit a rich diversity of morphological phenotypes, electrophysiological properties, and gene expression patterns. Investigating how these different characteristics are related at the single-cell level has been challenging, and most existing taxonomies of neurons are based on morphological or transcriptomic data alone. Patch-seq combines whole-cell patch-clamp recording, biocytin statining, and single-cell RNA-sequencing (scRNA-seq), enabling the comprehensive profiling of single neurons across all three modalities. We used Patch-seq to analyze neurons in the primary motor cortex (MOp) of adult mice, as part of the Brain Initiative Cell Census Network (BICCN) project. We performed recordings in all cortical layers using various Cre-line mice, allowing us to cover most of the ~80 transcriptomic types identified from parallel work at the Allen Institute for Brain Science using dissociated neurons. We collected data from over 1000 cells, with ~100% having detailed electrophysiological and >90% with high quality transcriptomic chacterization. We recovered the morphology for more than 60% of these neurons. This includes excitatory neurons, as well as *Pvalb+*, *Sst+*, *Vip+*, and *Lamp5+* interneurons. Using this data set, we were able to provide a morphological and electrophysiological signature for most transcriptomic types. Conversely, for most morphological types known from the literature we identified their corresponding transcriptomic types. So far, our preliminary analysis suggests that multiple transcriptomic types have similar morphological and electrophysiological features. Thus multimodal profiling is a critical step towards understanding the principles of what constitutes a cell type in the brain and creating a census of cell types.

Disclosures: F. Scala: None. **M. Bernabucci:** None. **D. Kobak:** None. **J.R. Castro:** None. **Y. Bernaerts:** None. **S. Laturnus:** None. **L. Hartmanis:** None. **C.R. Cadwell:** None. **E. Miranda:** None. **D. Ramsköld:** None. **Z. Yao:** None. **O. Penn:** None. **K.R. Tolias:** None. **B. Tasic:** None. **R. Sandberg:** None. **X. Jiang:** None. **H. Zeng:** None. **P. Berens:** None. **A.S. Tolias:** None.

Poster

250. Transcriptomic and Genomic Analyses

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 250.12/AA37

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: CAMH StartUp Funds

Title: Predicting electrophysiological, morphological, and computational features from single-cell transcriptomics data

Authors: *S. TRIPATHY;
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Abstract: Despite massive increases in the scale and applicability of single-cell genomics, translating cell-type specific gene expression profiles to cellular phenotypes remains a challenge. Here, we describe our approaches for identifying cross-modal relationships between cell type-specific transcriptomes and multiple features of neuronal activity, including electrophysiological, morphological, and synaptic profiles as well as the parameters of single neuron computational models. Our methods utilize machine learning algorithms to learn multi-variate relationships between the expression of multiple genes and downstream cellular phenotypes. By further assessing the replicability of these relationships in publicly-accessible Patch-Seq datasets, where multi-modal measurements be assayed from the same single cell, we can further test whether these relationships generalize within individual types. Lastly, we demonstrate that these relationships can be used to infer cell type-specific phenotypic alterations, such as those in aging or neuropsychiatric disorders, directly from gene expression alterations alone.

Disclosures: S. Tripathy: None.

Poster

250. Transcriptomic and Genomic Analyses

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 250.13/AA38

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: NIH Grant U19MH114830

Title: High-resolution transcriptomic cell type atlas reveals shared and distinct signatures across all cortical and hippocampal areas of mouse brain

Authors: *Z. YAO[1], L. T. GRAYBUCK[4], T. NGUYEN[5], K. A. SMITH[7], C. VAN VELTHOVEN[8], N. DEE[8], D. BERTAGNOLLI[8], J. GOLDY[8], O. FONG[8], B. P. LEVI[5], S. M. SUNKIN[2], M. J. HAWRYLYCZ[9], B. TASIC[6], H. ZENG[3];
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Abstract: Building high-quality atlas of brain cell types is a critical step towards understanding the relationship between brain structure and function. As part of the BRAIN Initiative Cell Census Network (BICCN), we have made steady progress towards building state-of-the-art single-cell transcriptomic cell type atlas from all brain regions of adult male and female mice, using highly standardized experimental and computational pipelines. We have currently completed data collection and preliminary analysis for cortical and hippocampal areas using both Smart-seq and 10x platforms. For the Smart-seq dataset, **74,985**cells collected from 20 major

regions and 79 more finely dissected areas are grouped into 291 clusters (92 GABAergic clusters, 179 Glutamatergic clusters and 20 non-neuronal clusters), whereas**1,093,785** cells collected from 18 major regions by 10x platform are grouped into 372 clusters (102 GABAergic clusters, 244 Glutamatergic clusters and 26 non-neuronal clusters). Joint clustering of both datasets using in-house developed computational pipeline (R package suite Scrattch) identified 396 clusters (127 GABAergic clusters, 244 Glutamatergic clusters and 25 non-neuronal clusters), with 374 clusters shared by both platforms. The Smart-seq and 10x datasets provide highly consistent cell type taxonomy. As we reported previously in a published study based on two cortical regions, inhibitory cell types are shared across all cortical regions, but also include additional hippocampus-specific clusters. All glutamatergic cell types show strong regional specificity, although the gene expression differences among neighboring cortical regions present themselves as continua for many cell types. The principal dimensions of such graded variation largely recapitulate the 3D spatial distribution of brain regions. Hippocampal formation regions, including entorhinal cortex, subiculum and hippocampus, can be clearly distinguished from the cortical areas, and critical transitional types between cortical and hippocampal glutamatergic cell types have been identified. Overall, the expanded transcriptomic cell type taxonomy reveals conserved organizational rules for molecularly defined cell types across all cortical and hippocampal areas, while sheding new light on the general principles for cortical and hippocampal regional diversity.

Disclosures: Z. Yao: None. **L.T. Graybuck:** None. **T. Nguyen:** None. **K.A. Smith:** None. **C. van Velthoven:** None. **B.P. Levi:** None. **N. Dee:** None. **D. Bertagnolli:** None. **J. Goldy:** None. **O. Fong:** None. **S.M. Sunkin:** None. **M.J. Hawrylycz:** None. **B. Tasic:** None. **H. Zeng:** None.

Poster

250. Transcriptomic and Genomic Analyses

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 250.14/AA39

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: BRAIN Initiative grant U19MH114830

Title: Transcriptomic cell types of the mouse thalamus

Authors: *C. T. J. VAN VELTHOVEN[1], Z. YAO[2], L. T. GRAYBUCK[1], T. NGUYEN[1], K. A. SMITH[3], N. DEE[3], D. BERTAGNOLLI[3], J. GOLDY[3], O. FONG[1], B. P. LEVI[4], S. M. SUNKIN[5], M. HAWRYLYCZ[6], B. TASIC[1], H. ZENG[7];
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Abstract: In the mammalian brain, thalamus is a major relay center that conveys motor and sensory signals to the cortex. Recent advances in single-cell RNA-sequencing provide a powerful approach to identify the diversity of cells based on their gene expression profile. To this end we have analyzed ~120,000 cells dissected from four broad thalamic regions, covering the complete thalamus using droplet-based single-cell RNA-sequencing (10x Genomics platform). We derived a transcriptomic taxonomy of cell types by iterative, bootstrapped weighted gene coexpression network analysis. We identified 182 neuronal cell types in mouse thalamus, of which 63 are GABAergic and 119 are glutamatergic neurons. To allow us to distinguish cell types based on their location within thalamus we performed fine dissections of specific thalamic nuclei and processed ~11,000 cells using well-based single-cell RNA-sequencing (Smart-seq v4). These data allowed us to identify 89 neuronal cell types of which 26 are GABAergic and 63 are glutamatergic. Joint analysis of the two datasets enabled us to pinpoint the spatial location of the newly identified cell types. We confirmed gene expression patterns and cell type distribution within thalamus using RNA *in situ* hybridization and confirmed correspondence of transcriptomic signatures with neuronal projection specificity by combining retrograde labeling and single-cell RNA-sequencing. Our study provides a high resolution transcriptomic taxonomy of cell types within mouse thalamus.

Disclosures: C.T.J. van Velthoven: None. **Z. Yao:** None. **L.T. Graybuck:** None. **T. Nguyen:** None. **K.A. Smith:** None. **N. Dee:** None. **D. Bertagnolli:** None. **J. Goldy:** None. **O. Fong:** None. **B.P. Levi:** None. **S.M. Sunkin:** None. **M. Hawrylycz:** None. **B. Tasic:** None. **H. Zeng:** None.

Poster

250. Transcriptomic and Genomic Analyses

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 250.15/AA40

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: R01MH112739

Title: Defining cellular diversity in the caudal region of the hypothalamus through single cell transcriptomic analysis

Authors: L. E. MICKELSEN[1], M. BOLISETTY[2], P. ROBSON[3], *A. C. JACKSON[1]; [1]Dept. of Physiol. and Neurobio., Univ. of Connecticut, Storrs, CT; [2]Bristol-Myers Squibb, Pennington, NJ; [3]The Jackson Lab. for Genomic Med., Farmington, CT

Abstract: The caudal region of the hypothalamus is home to distinct subregions, including the medial and lateral mammillary nuclei as well as the tuberomammillary, premammillary and retromammillary nuclei. These hypothalamic subregions are known to regulate diverse

physiological and behavioral functions such as wakefulness, memory and reproduction. In particular, the medial and lateral mammillary nuclei give rise to the mammillothalamic tract (MT), a major hypothalamic projection to the anterior thalamic nuclei. Neuronal cell type diversity within the caudal hypothalamus is poorly understood. To address this question, we employed a single cell transcriptomic approach to classify molecularly distinct neuronal and non-neuronal cell types in the caudal hypothalamus. Specifically, we used droplet-based single cell RNA sequencing (scRNA-seq) of isolated cells from microdissected caudal hypothalamic slices collected from juvenile male and female mice. We then used unsupervised cluster analysis to classify diverse populations of putative non-neuronal and neuronal cell types. Among neuronal clusters in the caudal hypothalamus, we found that a majority expressed markers of glutamatergic transmission (Slc17a6), while a minority expressed GABAergic markers (Slc32a1, Gad1 and Gad2). In addition, we identified a distinct population of putative histaminergic neurons, characterized by discriminatory genes including Hdc, Slc18a2 and Gad1. Generally, we found that caudal hypothalamic neuronal clusters could be discriminated by suites of markers that include neuropeptides, transcription factors, synaptic proteins and calcium-binding proteins. We validated differentially expressed genes in a selection of putative neuronal populations through fluorescence *in situ* hybridization (FISH). Focusing on the mammillary nuclei, we found cellular diversity among neuronal populations that give rise to the MT and that transcriptionally distinct clusters broadly aligned with neuroanatomical compartments. This single cell transcriptomic analysis of putative cell types in the caudal hypothalamus provides a resource for advancing our understanding of the circuit-level mechanisms underlying the diverse functions of caudal hypothalamic circuits in health and disease.

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Poster

250. Transcriptomic and Genomic Analyses

Location: Hall A

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Program #/Poster #: 250.16/AA41

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: NIH Grant 5 U01 MH114812-02

Title: Surveying cellular diversity in the adult human whole brain

Authors: K. SILETTI[1], ***R. D. HODGE**[2], T. E. BAKKEN[2], S.-L. DING[2], A. YANNY[2], T. CASPER[2], N. DEE[2], D. HIRSCHSTEIN[2], L. HU[1], N. JORSTAD[2], P. LONNERBERG[1], S. MOK[2], J. NYHUS[2], N. SHAPOVALOVA[2], S. M. SUNKIN[2], E. LEIN[2], S. LINNARSSON[1]; [1]Karolinska Inst., Stockholm, Sweden; [2]Allen Inst. for Brain Sci., Seattle, WA

Abstract: The human brain is composed of a dense network of interconnected, spatially localized cells that are responsible for executing varied cognitive functions and movement. A complete understanding of the diversity of cell types that comprise different brain regions has been difficult to achieve in human due to inadequate access to brain tissues and low-throughput techniques for characterizing cells. Recent advances in single-nucleus transcriptomics have enabled comprehensive analyses of molecularly-defined cell types in banked frozen human brain specimens and revealed a high degree of cellular diversity in human brain, including more than 50 cell types in a single area of neocortex. Here, we deeply sampled cellular diversity across ~100 human brain regions by profiling the nuclear transcriptomes of more than 200,000 cells using single nucleus RNA-sequencing. These regions were sampled from major brain structures with distinct developmental origins, including the telencephalon, diencephalon, midbrain, and hindbrain, and with a focus on cellular variation across ~30 cortical areas. We performed detailed annotations of regions of interest in control postmortem adult human brain specimens and isolated nuclei using fluorescence-activated cell sorting with NeuN antibody staining to distinguish neuronal and non-neuronal nuclei. Isolated nuclei were processed for RNA-sequencing using high throughput droplet-based sequencing technology. We identify a diverse of set cell types, many of which are regionally-specific, and define an initial hierarchical taxonomy of neuronal and non-neuronal cell types that span the whole human brain.

Disclosures: K. Siletti: None. **R.D. Hodge:** None. **T.E. Bakken:** None. **S. Ding:** None. **A. Yanny:** None. **T. Casper:** None. **N. Dee:** None. **D. Hirschstein:** None. **L. Hu:** None. **N. Jorstad:** None. **P. Lonnerberg:** None. **S. Mok:** None. **J. Nyhus:** None. **N. Shapovalova:** None. **S.M. Sunkin:** None. **E. Lein:** None. **S. Linnarsson:** None.

Poster

250. Transcriptomic and Genomic Analyses

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 250.17/AA42

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: NIH R21 NS106447
NIH 5UL1TR001105
HCA-A-1704-01747 Chan Zuckerberg Initiative
James S. McDonnell Foundation Understanding Human Cognition Scholar Award 220020467
T32HL139438

Title: Resolving cellular and molecular diversity along the hippocampal anterior-to-posterior axis in humans

Authors: *F. AYHAN[1], A. KULKARNI[1], C. DOUGLES[1], B. C. LEGA[2], G. KONOPKA[1];
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Abstract: The hippocampus is critical for diverse brain functions including learning, memory, cognition, and emotional processing. Anatomically, the hippocampus runs along a longitudinal axis, posterior-to-anterior in primates (corresponding to dorsal-to-ventral in rodents). The structure, function, and connectivity vary along this axis. The posterior hippocampus is primarily implicated in cognitive functions whereas; the anterior hippocampus mediates behaviors related to stress response and emotional regulation. Variability in gene expression across the hippocampal axis has been studied in rodents however molecular and cellular heterogeneity across the hippocampal axis in human is unknown. To better understand cellular composition and molecular diversity along the hippocampal long axis in humans and define distinct molecular signatures corresponding to various functional domains we performed single-nuclei RNA-sequencing on surgically resected human anterior and posterior hippocampus. Analysis of ~90,000 nuclei revealed distinct proportions of excitatory neuronal cell-types in anterior and posterior hippocampus. Moreover, similar cell types from these regions show gene expression differences suggesting distinct roles. Our data illuminate a region- and cell-type specific transcriptional landscape within the hippocampus. Future studies will functionally connect the cell-types and genes that underlie the distinct functions of anterior and posterior hippocampus.

Disclosures: F. Ayhan: None. **A. Kulkarni:** None. **C. Dougles:** None. **B.C. Lega:** None. **G. Konopka:** None.

Poster

250. Transcriptomic and Genomic Analyses

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 250.18/AA43

Topic: I.02. Systems Biology and Bioinformatics

Support: NIH Grant 1DP1DA046585

Title: Nested tree probabilistic graphical models to trace the evolutionary histories and spatial patterns across neural cell types

Authors: *M. KLEYMAN[1], J. HE[2], B. E. OZTURK[3], M. WIRTHLIN[1], L. BYRNE[3], W. R. STAUFFER[2], A. R. PFENNING[1];
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Abstract: Recent advances in single cell genomics have provided new insights into the transcriptional state of neural cell types. Despite these advances, we still know little about how

these cell type-specific gene expression patterns evolve across closely and distantly related species or how they vary across different brain regions. To trace the evolutionary and spatial patterns of gene expression levels across cell types, we have developed a novel machine learning method called a nested tree probabilistic graphical model that can learn hierarchical changes of gene expression across both species and spatial location based on single cell RNA-seq data. Our method leverages the concepts of a species or spatial hierarchy with the evolutionary theory of maximum parsimony to extract meaningful gene expression changes across multiple single cell datasets. We applied our method to analyze the evolutionary histories of cell types in the retina across primates and rodents and the spatial patterns of the cell types in the primate basal ganglia. Our method was able recapitulate known cell types and their associated marker genes, characterize the heterogeneity of each cell type that corresponded to biological pathways, and identify across what stage of a species, spatial, or cell type hierarchy reproducible gene expression changes occur. It also allows us to make inferences about gene function and co-interaction in neural cell types based on gene coevolution or spatial co-locality. Through these analyses our work significantly advances the understanding of the transcriptional state of neuronal cell types in terms of their evolutionary of spatial contexts.

Disclosures: **M. Kleyman:** None. **J. He:** None. **B.E. Ozturk:** None. **M. Wirthlin:** None. **L. Byrne:** None. **W.R. Stauffer:** None. **A.R. Pfenning:** None.

Poster

250. Transcriptomic and Genomic Analyses

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 250.19/AA44

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: CREST/JST

Title: A novel state-of-the-art system for the single cell transcriptome analysis based on planar patch clamp

Authors: ***S. ISHIGAKI**[1], H. UNO[2], Z.-H. WANG[2], Y. UKITA[3], R. BHARDWAJ[4], P. T. TUE[4], S. IWABUCHI[5], S. HASHIMOTO[5], T. OKA[6], K. KAWAHARA[6], Y. TAKAMURA[4], T. URISU[2], G. SOBUE[1];
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Abstract: The recent advance in the technologies of single cell analysis has brought a large impact. However, most of the devices for single cell analysis are based on microfluidics or

droplet methods which have limitations of application in neuroscience due to technical difficulties in cell separation.
The goal of this research is to develop a technical base for the comprehensive analysis of biomolecules in single cells on 2D plane, such as primary neurons, in keeping with the positional information of the cells. Furthermore, the device equips both the planar patch clamp and the micro pore which allows us to analyse the neuronal activity and its transcriptome profiles simultaneously.
For the purpose, we developed the array of single-cell-analysis-units which extract the cytoplasm using micro actuators or syringe pumps, to hand out the extract to mRNA-seq analysis. We analyzed single cells positioned on the micro pore of the planar patch clamp chip by suction through the micro pore. The transcriptome profiles of single cells were analyzed by qPCR and RNA-seq analysis using next-generation sequencing (NGS). We screened several different conditions of negative pressures and confirmed that the majority of the mRNA in the single cell was recovered through the pore with little contamination when the cell was pretreated with buffer containing detergent followed by suction with week negative pressure (1 kPa).
Furthermore, rat primary hippocampal neurons were successfully cultured on the developed planar patch clamp chip for a month after lentivirus infection. Then, the single-cell-extraction of rat primary hippocampal neurons was performed at DIV14 and DIV23 using the developed device chip. The transcriptome profiles were successfully obtained from single neuronal extracts using NGS.
The developed planar chip device will further enable us to analyze the fingerprints of single cells in heterogeneous tissue culture systems such as neuron-glia co-culture models of neurological diseases in which the non-cell-autonomous mechanism is linked to disease pathogenesis.

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Poster

250. Transcriptomic and Genomic Analyses

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NIH Grant EY02858
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The Mathers Foundation
The Champalimaud Foundation

The Good Ventures Foundation

Title: Single cell RNAseq in neuronal tissues: Impact of enzymatic dissociation on gene expression

Authors: *M. K. DREWS[1], M. B. CHEN[2,6], I. A. MARIN[3], S. R. QUAKE[4,6], C. J. SHATZ[5]; [1]Neurosciences Grad. Program, [2]Bioengineering, [3]Dept. of Biol., [4]Bioengineering and Applied Physics, [5]Biol. and Neurobiology, BioX, Stanford Univ., Stanford, CA; [6]Chan Zuckerberg Biohub, San Francisco, CA

Abstract: Single cell RNA sequencing (scRNAseq) is a very popular method in neuroscience for interrogating the cellular compositions of different brain regions. However, a potential limitation of scRNAseq in tissues like the brain is that these tissues do not exist naturally as single cells, but instead are highly interconnected multicellular organs. To generate single cells from intact brain tissue, a fairly lengthy enzymatic dissociation process is performed on still living cells. This harsh procedure raises concerns as to what impact enzymatic dissociation has on gene expression as well as the ensuing bioinformatic clustering analyses. To address these issues, we have taken forebrain tissue from adult mice and divided it into the two hemispheres. One hemisphere was immediately flash frozen for immediate RNA extraction, while the other hemisphere was dissociated into single cells, as one would do for scRNAseq, before RNA was harvested in bulk. RNA derived from these two conditions was then sequenced and differential expression analysis performed. We observe that even at very stringent significance thresholds, approximately 15% of the transcriptome is perturbed following enzymatic dissociation as compared with rapid homogenization. Included among perturbed upregulated genes are inflammatory and damage response genes such as HSPa1a and KLF2, as well as neuronal immediate early genes such as Fos and Jun. Included among the downregulated genes are synaptic organizing proteins and synaptic transmission and signaling proteins such as CAMK2a and KCNAB2. When significantly perturbed genes were removed from single cell clustering analysis performed on existing scRNAseq data from mouse cortex prepared in the same way, partially different clusters were obtained as compared to when the genes were included (Rand = 0.77), indicating that expression of these dissociation-perturbed genes may be influencing single cell clustering as an artifact of preparation for scRNAseq rather than due to inherent biological variation. These observations reveal important caveats regarding the use of scRNAseq for brain cells, and stress the importance of validating candidates using independent methods.

Disclosures: M.K. Drews: None. **M.B. Chen:** None. **I.A. Marin:** None. **S.R. Quake:** None. **C.J. Shatz:** None.

Poster

250. Transcriptomic and Genomic Analyses

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 250.21/BB2

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Title: Effect of post-mortem interval on ribosome-profiling in mouse frontal cortex

Authors: *A. D. BROWN[1], L. FIORI[2], J. F. THÉROUX[2], G. TURECKI[2];
[1]Human Genet., McGill Univ., Montréal, QC, Canada; [2]Douglas Mental Hlth. Univ. Inst., Montréal, QC, Canada

Abstract: Introduction: Ribosome-Profiling (or Riboseq) is a RNA sequencing technique that captures and profiles ribosomal footprints (RPFs): the <30bp fragments of the mRNA that are contained under ribosomes during active translation (known as the translatome). Discrepancies between the translatome and transcriptome (or genome) that are identified by riboseq are likely sites of epigenetic regulation, making it a prime methodology for the study of epigenetic regulation in diseases of the central nervous system. Ideally, to study these diseases through riboseq, libraries would be constructed from post-mortem brain tissue (either human or from an animal model). However, successful RPF capture can easily be affected by post-mortem interval (PMI) in these tissues due to translation processes continuing for a brief period post-mortem, and through degradation of ribosomes and RPFs during the PMI. Due to this, the effect of PMI on the feasibility of RPF capture from post-mortem brain has substantial implications for its efficacy as an epigenetic methodology. This is why we have endeavored to quantify RPF capture and sequencing quality at various PMIs in a mouse model. We hypothesize that total reads and potentially RPF fragment length will show a significant negative correlation to PMI.
Methods: An adapted version of the conventional riboseq procedure (specialized for post-mortem brain tissue) was used to extract the RPFs from the homogenized frontal cortex tissue. Subject groups included controls (immediately flash frozen tissue), and PMI samples left at room temperature for 0.5,3,6 and 12 hours before being refrigerated for 24 hours, and then flash frozen. Concurrently, RNAseq was completed in the same mouse PMI samples, to measure efficacy of RPF capture specifically. Both RPF and total RNA libraries were constructed using SMARTer® smRNA-Seq Kit, and sequenced via Miseq V3 150 cycle sequencing. Evaluation of RPF capture efficacy includes measures of coding enrichment, triplicate periodicity, and read alignment.
Results: Data analysis is ongoing, but preliminary results indicate a triplicate periodicity profile typical of RPFS in the mouse RPF samples compared to the total RNA samples, and a significant fraction of coding regions captured in the RPF samples. Fragment size, coding region fractions, and triplicate periodicity profiles seem to be consistent across PMIs, potentially indicating that differences in PMI at these intervals does not have a significant effect on RPF capture feasibility,

indicating that PMI differences of this magnitude likely do not adversely affect the feasibility of performing riboseq in post-mortem brain tissue, contrary to expectation.

Disclosures: **A.D. Brown:** None. **L. Fiori:** None. **J.F. Théroux:** None. **G. Turecki:** None.

Poster

250. Transcriptomic and Genomic Analyses

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 250.22/BB3

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: CONACyT Postdoctoral Fellowship no. 2018-000005-01NACV-00163

Title: A novel technique for analyzing brain microvascular endothelial cell DNA methylation in mouse cerebral cortex and hippocampus

Authors: *D. COLIN-CASTELAN, S. ZAINA;
Med. Sci. Dept., Univ. de Guanajuato, Leon, Mexico

Abstract: CChanges in DNA methylation generate stable transcriptional patterns in the brain, both in physiological and pathological conditions. Given that distinct cognitive functions are often associated with specific brain regions and/or cell types, analyzing the DNA methylation patterns in different brain structures and cell phenotypes is essential. Brain microvascular endothelial cells (BMEC) are of special interest because they play complex roles beyond their blood-brain barrier function, including the formation of vascular neurogenic niches and the clearance of neurotoxic peptides from the brain parenchyma. However, primary BMEC methylation patterns have not yet been explored, partially due to the technical difficulty of isolating enough genomic BMEC DNA for bisulfite conversion and analysis, especially when trying to study the vascular methylation patterns from distinct brain regions. This study presents a novel methodology for the isolation, bisulfite conversion and analysis of genomic BMEC DNA from cerebral cortex and hippocampus from individual mice. Briefly, the proposed methodology consists of dissecting the brain areas of interest, isolating their BMEC by filtration and centrifugation, isolating the genomic DNA, performing bisulfite conversion and analyzing by pyrosequencing. In order to assess whether the BMEC isolation protocol indeed yielded purified endothelial cells, we analyzed the purified cell samples by fluorescence histochemistry using the endothelial cell marker *B. simplicifolia* I Isolectin B4. Once we confirmed that we only obtained BMEC, we isolated the genomic DNA from hippocampus (804.7±245.4 ng) and cerebral cortex endothelium (1844.4±554.78 ng). Subsequently, we performed the bisulfite conversion of the isolated DNA and finally amplified the B1 repetitive element sequence by PCR and successfully analyzed the product by pyrosequencing. This new technique allows the study of vascular epigenetic changes within specific brain regions without the need for sample pooling, thus

opening the door for a better understanding of the vascular role in brain function, development, and disease.

Disclosures: D. Colin-Castelan: None. **S. Zaina:** None.

Poster

251. Anatomic Methods: Circuit Tracing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 251.01/BB4

Topic: I.03. Anatomical Methods

Support: .

Title: Magnetic resonance imaging of the brain of a diprotodontid marsupial, the quokka (setonix brachyurus)

Authors: *J. THITTAMRANAHALLI KARIYAPPA, A. BONGERS, K. W.S.ASHWELL; Anat., Univ. of New South Wales, Sydney, Australia

Abstract: The diversity of the diprotodontids provides an excellent opportunity to study how a basic marsupial cortical plan has been modified for the needs of the mammals living in the different habitats. Very little is known about the connections of the cerebral cortex with the deep brain structures (basal ganglia and the thalamus) in this evolutionarily significant group of mammals. The injured animal was painlessly euthanized with Lethobarb and the brain removed at Taronga Zoo, Sydney. The brain was fixed in formaldehyde, immersed in Fomblin and scanned. High resolution anatomical and Diffusion Tensor Imaging was performed using a 9.4-T Bruker BioSpec 94/20 Avance III MRI system (Bruker, Ettlingen, Germany) located at UNSW in Sydney. This is the first comprehensive account of MRI mapping of brain connections in a diprotodontid marsupial. We were able to identify corticostriate connections between the frontal association and dorsomedial isocortex and the head of the caudate, and between the lateral somatosensory cortex and the putamen. We were also able to follow the olfactory and limbic connections by tracing fibers in the fornix, cingulum, intrabulbar part of the anterior commissure and lateral olfactory tract. There was segregation of fibers in the anterior commissure such that olfactory connections passed through the rostroventral part and successively more dorsal cortical areas connected through more dorsal parts of the commissure. Our findings confirm a common pattern of cortical connectivity in therian mammals, even where brain expansion has occurred independently in diverse groups.

Disclosures: J. Thittamranahalli kariyappa: None. **A. Bongers:** None. **K. W.s.ashwell:** None.

Poster

251. Anatomic Methods: Circuit Tracing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 251.02/BB5

Topic: I.03. Anatomical Methods

Support: MOST Grant 105-2320-B-002-055-MY3

Title: Concomitant top-down innervations from the prefrontal cortex onto functionally connected interneurons and noradrenergic neurons in locus coeruleus

Authors: *Y.-S. KUO[1], M.-Y. MIN[1], C.-C. KUO[1], J.-C. HSIEH[1], H.-W. YANG[2];
[1]Dept. of Life Science, Natl. Taiwan Univ., Taipei, Taiwan; [2]Dept. of Biomed. Sci. Chung-Shan Med. Univ., Taichung, Taiwan

Abstract: In this study, we question whether there were local interneurons (IN) able to intergrate cortical signal to noradrenergic (NA) neurons in locus coeruleus (LC). LC-NA neurons are major norepinephrine supply to CNS through global axonal projections. LC-NA system plays important roles in behavior, such as promoting wakefulness and vigilance. Evidences from recent studies have suggested that LC-NA system serves as a temporary filter that allows outcome of decision made by high cognitive cortical areas to facilitate behavior through increasing the gain of neuronal network with precise timing by undergoing LC phasic activity. Prefrontal cortex (PFC) plays significant roles in evaluation of reward and in cognitive functions overlapping with those attributed to LC-NA system. Previous studies in monkeys and rats have shown that PFC projects to LC with axonal terminals identified in peri-LC region, where dendrites of LC-NA neurons are located. Since numerous local interneurons are also located in peri-LC region, we examined if the IN in peri-LC and LC-NA neurons received top-down innervations from the PFC. We produced an adeno-associated virus (AAV) carrying a double-floxed inverted open reading frame of wheat-germ-agglutinin (WGA), a commonly used trans-neuronal tracer. The AAV was injected into LC of offspring of TH^{cre} mouse (tyrosine hydroxylase.) crossed with GAD^{GFP} (glutamic acid decarboxylase) mouse. This would result in selective expression of WGA in LC-NA neurons specifically in LC nucleus. Using immunohistochemistry method, we observed TH and WGA- immunoreactive (ir) within LC as expected, but also a few neurons that did not show TH-ir but WGA-ir in the LC and the surrounding areas. Given leak-out expression of cre in other cells than in LC-NA neurons was very limited in the TH-cre mouse, we reason that these IN were labelled as result from trans-neuronal transport of WGA from LC-NA neurons. These interneurons were located predominantly in medial aspect of peri-LC region, the gap between LC proper and the adjacent Barrington nucleus. Among the IN, 15% are found to express GAD and 40 % express FoxP2. To confirm whether the IN received top-down cortical inputs, we repeat above experiments with an additional injection of AAV carrying reading frame of Crimson into

the PFC for anterograde tracing. We found that PFC fibers not only contact on LC neurons but also on the IN (non-TH-ir but WGA-ir) expressing GAD and FoxP2. Together, these results do support our arguments that there are local excitatory and inhibitory IN in medial peri-LC have functional connections with LC-NA neurons and could integrate PFC inputs onto LC-NA neurons.

Disclosures: Y. Kuo: None. **M. Min:** None. **C. Kuo:** None. **J. Hsieh:** None. **H. Yang:** None.

Poster

251. Anatomic Methods: Circuit Tracing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 251.03/BB6

Topic: I.03. Anatomical Methods

Support: NIH: 5R01NS089770
NIH: 1R21NS104868
NIH: 1F30MH116650

Title: The output network of the zone of uncertainty

Authors: *R. KERY[1,2], L. CHEN[1], H. LIU[1], Q. XIONG[1], S. GE[1];
[1]Dept. of Neurobio. and Behavior, Stony Brook Univ., Stony Brook, NY; [2]Med. Scientist Training Program, Stony Brook Univ. Sch. of Med., Stony Brook, NY

Abstract: The zona incerta is a brain region between thalamus and hypothalamus. Besides its potential importance in many brain behaviors, emerging evidence has shown that deep brain stimulation of the caudal zona incerta is superior to stimulating the subthalamic nucleus in treating patients with Parkinson's Disease. In contrast to these behavioral or clinical roles, the network of the zona incerta remains poorly understood. To map out synapse outputs of zona incerta, we recently developed a novel anterograde trans-synaptic tracing method, which allows us to mark the cell-type specific synapse targets. Using this method, we systematically analyze the synapse output of zona from anterior to posterior brain regions including spinal cord. Importantly, using a *cre recombinase*-dependent version of our tracing construct, we have mapped the output projections of three of the most common neural subtypes found in the zona: the somatostatin, parvalbumin, and nitric oxide cell populations. These mapping data for the first time provides us a clear map of the synaptic output of zona incerta, which will provide much-needed insight for further exploration of zonal circuitry.

Disclosures: R. Kery: None. **L. Chen:** None. **H. Liu:** None. **Q. Xiong:** None. **S. Ge:** None.

Poster

251. Anatomic Methods: Circuit Tracing

Location: Hall A

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Program #/Poster #: 251.04/BB7

Topic: I.03. Anatomical Methods

Support: R01MH118257
P41EB027061
MnDrive Brain Conditions
T32DA007234
NARSAD Young Investigator Award

Title: Diffusion tractography at 10.5T in nonhuman primates

Authors: *M. D. GRIER[1], J. ZIMMERMAN[2], S. MOELLER[2], G. ADRIANY[2], R. LAGORE[2], N. HAREL[2], E. YACOUB[2], R. ZHANG[2], C. LENGLET[2], K. UGURBIL[2], S. R. HEILBRONNER[1];
[1]Neurosci., [2]Ctr. for Magnetic Resonance Res., Univ. of Minnesota Twin Cities, Minneapolis, MN

Abstract: Diffusion weighted magnetic resonance imaging (dMRI) utilizes specific MRI sequences and advanced data processing that allow for the mapping of water diffusion throughout biological tissue. One application of dMRI is white matter tractography, a method that allows for the visualization of white matter pathways and estimation of connectivity strength between brain regions *in vivo* and non-invasively. Historically, significant limitations of dMRI have meant that tractography is only weakly correlated with true anatomical connectivity (as estimated with tract-tracing). For example, one such limitation is the comparatively large voxel size compared to the size of white matter tracts present in the brain. Additional limitations include the long scan time normally required to collect high-resolution dMRI data. We present data that reduces these limitations using high-field imaging in nonhuman primates at a unique 10.5T scanner and advanced processing steps on subjects also undergoing traditional tract-tracing studies. These data are acquired at .58 mm resolution over approximately one hour. We discuss the optimization process for overcoming intrinsic problems with increased resolution including reduced signal-to-noise ratio (SNR) and an apparent linear drift over the long duration scans required for high resolution dMRI. Utilizing a variety of freely available software combined with a new denoising algorithm, we have generated tractography maps that recapitulate known white matter pathways better than traditional approaches when compared to previously published tract tracing data. We plan to advance these studies by utilizing within-individual neural tract tracing experiments in a series of highly novel studies comparing *in vivo*

dMRI results with tract tracing data acquired from the same subjects. This will provide a unique opportunity to validate and improve dMRI.

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Poster

251. Anatomic Methods: Circuit Tracing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 251.05/BB8

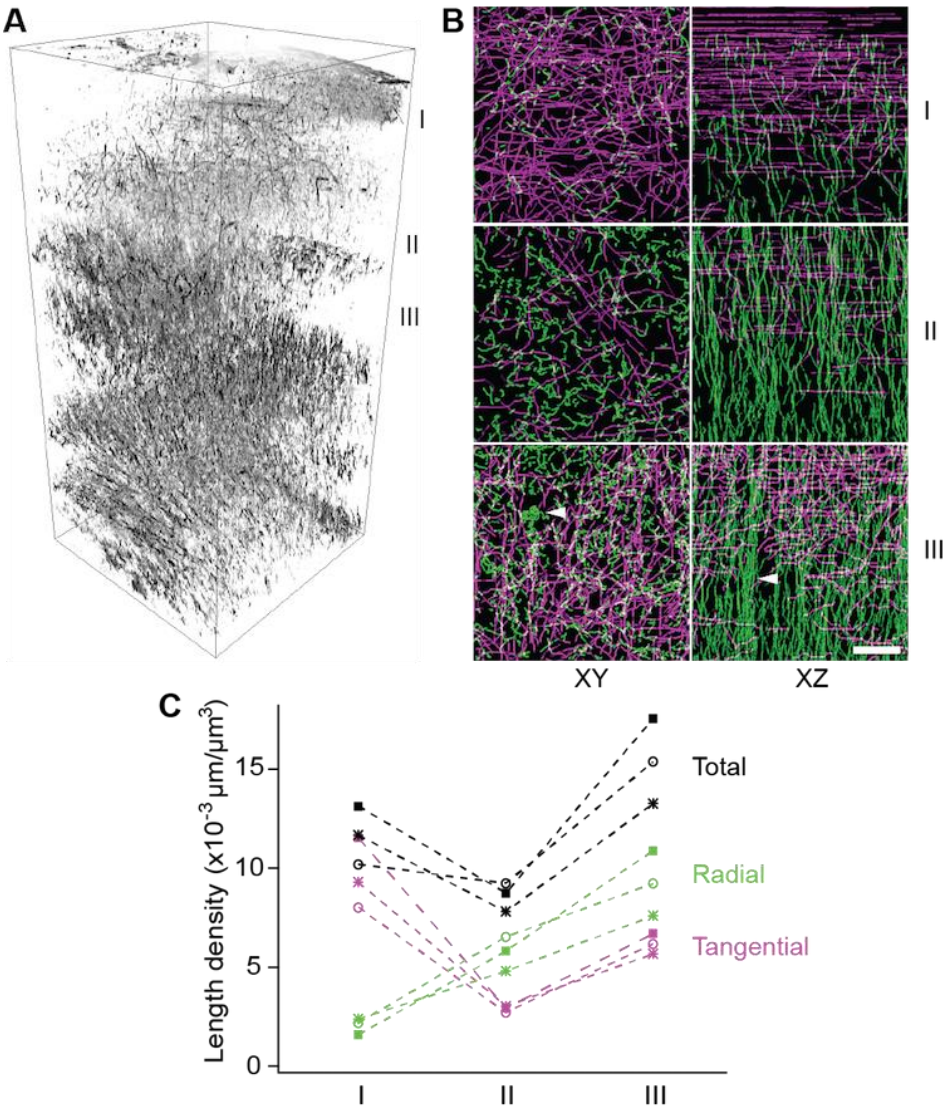
Topic: I.03. Anatomical Methods

Support: NIH Grant GM121198

Title: A method to measure myeloarchitecture of the murine cerebral cortex *in vivo* by intrinsic third-harmonic generation

Authors: M. REDLICH, ***H. LIM**;
Hunter College, CUNY, New York, NY

Abstract: A new label-free method is presented for measuring myeloarchitecture of the murine cerebral cortex in vivo and ex vivo. Growing evidence suggests that cortical myelination plays significant roles in neuronal plasticity and pathologies, such as multiple sclerosis (MS), but illuminating the mechanism requires longitudinal imaging of the same brains. Here we demonstrate imaging unlabeled myelinated fibers in a live mouse brain by third-harmonic generation (THG). Contrary to other label-free microscopies based on reflectance, fibers of all orientations could be visualized, i.e., radial and tangential to the pia, which is suitable for revealing the three-dimensional connectivity. The depth of THG imaging in an intact brain was approximately 200 μm, so the network of myelinated fibers could be captured into layers 2/3 in vivo. THG provides a novel base for reconstruction of morphology. Semi-automatic tracing of THG-positive axons unraveled the depth-dependent distribution of myelin lattice. Finally, a unique light property of THG was exploited for the estimation of the g-ratio. The demonstrated THG morphometry of the length density, orientation, and sheath thickness of cortical myelin could be useful for elucidating the function and how it is modulated during learning and diseases.



Disclosures: **M. Redlich:** None. **H. Lim:** None.

Poster

251. Anatomic Methods: Circuit Tracing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 251.06/BB9

Topic: I.03. Anatomical Methods

Title: Anatomical characterization of estrogen receptor expression in lateral habenula projection neurons in female rats

Authors: A. D. CHISHOLM, S. M. CLAYPOOL, P. R. SILVA, J. F. NEUMAIER, ***S. G. NAIR**;
Psychiatry and Behavioral Sci., Univ. of Washington, Seattle, WA

Abstract: The lateral habenula (LHb), an epithalamic nucleus, commonly known to be involved in the processing of aversive information, also mediates several estrogen-dependent behaviors in female rats. The effects of estrogen are primarily mediated by estrogen receptor subtypes α and β (ERα and ERβ). Our primary objectives were to determine if there are estrous cycle-dependent changes in ER expression in LHb neurons and to define the neuroanatomical organization of estrogen receptors in LHb projection neurons. To determine if there are cyclical, hormone-dependent changes in estrogen receptor plasticity in LHb neurons, brains from freely-cycling, female, Long-Evans rats were collected during the estrus cycle at proestrous, diestrus, metestrus and estrus and analyzed for the expression of ERα and ERβ in LHb neurons. Preliminary results indicate that the majority of ERα expressing neurons are located in the ventromedial aspect of the medial to caudal regions of the LHb. A few ERα containing neurons were detected in the rostral portions of the LHb. Analyses are currently underway to determine whether the expression of ERα and ERβ in LHb neurons vary as a function of the estrus cycle. To define the neuroanatomical organization of estrogen receptor expression in LHb projection neurons, rats were injected bilaterally with canine adenovirus (CAV2) expressing ZsGreen into either the ventral tegmental area (VTA), serotonergic dorsal raphe nuclei (DRN) or the the GABAergic rostromedial tegmental nucleus (RMTg), three targets of LHb output neurons. CAV2-ZsGreen vector efficiently infected axon terminals in the VTA, DRN and the RMTg and was retrogradely transported to neuronal cell bodies in the LHb where the ZsGreen transgene was expressed. RNAScope *in situ* hybridization studies are in progress to determine the co-localization of ZsGreen positive neurons in the LHb with ERα and ERβ.

Disclosures: A.D. Chisholm: None. **S.M. Claypool:** None. **P.R. Silva:** None. **J.F. Neumaier:** None. **S.G. Nair:** None.

Poster

251. Anatomic Methods: Circuit Tracing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 251.07/BB10

Topic: I.03. Anatomical Methods

Support: VA I21 RX002900
VA I01 RX001511
VA I01 RX001511
NIDA R01 DA042057
Wayne State University MD/PhD Program

Title: Quantifying stimulus-based neuronal activity in rat brain using high-resolution photoacoustic imaging

Authors: *J. I. MATCHYNSKI[1], R. MADANGOPAL[2], V. A. LENNON[3], R. MANWAR[4], K. J. KRATKIEWICZ[4], B. T. HOPE[5], S. A. PERRINE[4], A. C. CONTI[4], M. R. N. AVANAKI[6]; [1]Dept. of Behavioral Neurosciences, Wayne State Univ. Sch. of Med., Detroit, MI; [2]Natl. Inst. On Drug Abuse IRP, Baltimore, MD; [3]Natl. Inst. On Drug Abuse, Baltimore, MD; [4]Wayne State Univ., Detroit, MI; [5]Behav Neurosci, NIH/NIDA, Baltimore, MD; [6]Biomed. Engin., Wayne State, Detroit, MI

Abstract: Current functional imaging techniques, such as functional magnetic resonance imaging, rely upon activity-induced blood flood changes to neurons. This indirect measurement of neuronal activity inherently limits image resolution and specificity. However, advances in transgenic technology and photoacoustic (PA) methodology have offered new solutions to these limitations. Selective organic dyes and nanoparticles with high optical absorption in the near-infrared window, outside the range of which endogenous chromophores strongly absorb, can be valuable for generating a targeted signal with a high contrast-to-noise ratio. For example, virally-infected tumor cells can be made to express enzymes, such as LacZ, that are capable of cleaving colorless substrates into colored products in the presence of X-Gal. Therefore, since Fos is used as a marker of activated neurons, we propose using PA imaging to map activated neurons using a Fos-LacZ transgene reporter system in rats. Fusion of Fos with the lacZ gene gives active (Fos+) cells the ability to cleave pro-chromogenic substrates into PA-active dyes. In this study, we visualized neuronal activity PA signal in Fos-LacZ transgenic rats following two different stimulation methods.

We subjected Fos-LacZ rats to one of three conditions: Footshock, Cocaine bolus (one dose, 20 mg/kg), or home cage naïve. Ninety minutes after the stimulus presentation, the rats were injected in the medial prefrontal cortex (mPFC) with X-Gal. Brains were excised, then PA imaged *ex vivo*. We used an elevational scanning 18.5 MHz, 128 element L-22 linear array ultrasound transducer to record PA signal produced by pulsed laser illumination at both 690 nm and 850 nm nearly simultaneously through rapid scanning. Laser light was directed from a PA-optimized OPOTEK Phocus MOBILE laser by two fiber optic cable arrays placed on both sides of the probe to focus light approximately 1 cm beneath it. PA intensity within the mPFC of acquired images was quantified using ImageJ software.

We presently report quantified *in vivo* PA images of rat brains expressing X-Gal product prepared from footshocked, cocaine-treated, or naïve animals.

We discuss the feasibility of this reporter method for neuronal activity based on our acquired images, focusing on observed differences between stimulus-treated and naïve animals. With this technique, we propose a method of longitudinally monitoring activated (Fos+) neurons *in vivo* with high resolution and specificity.

Disclosures: J.I. Matchynski: None. **R. Madangopal:** None. **V.A. Lennon:** None. **R. Manwar:** None. **K.J. Kratkiewicz:** None. **B.T. Hope:** None. **S.A. Perrine:** None. **A.C. Conti:** None. **M.R.N. Avanaki:** None.

Poster

251. Anatomic Methods: Circuit Tracing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 251.08/BB11

Topic: I.03. Anatomical Methods

Support: JITRI Grant

Title: A novel sparse labeling strategy for brain-wide individual neuron reconstruction

Authors: *S. JIN[1], X. LI[2], S. TAO[3], A. LI[2], F. XU[3], H. GONG[2];
[1]Hust-Suzhou Inst. For Brainsmatics, Suzhou, China; [2]Wuhan Natl. Lab. For Optoelectronics, Wuhan, China; [3]Wuhan Inst. of Physics and Mathematics, Wuhan, China

Abstract: Full morphologies of individual neurons help us understand how information flow across axon,but the strategy to elucidate the complete morphology of single neuron whose axon project across the whole brain is still lacking. Here we developed a cocktail adeno-associated virus (AAV) packaging system by mixing different AAV plasmids together with specific ratios and co-packaging them into a single virus mixture to achieve sparse labelling. We applied the novel sparse labelling virus in several mouse brain regions and captured whole brain data by employing fMOST system. We reconstructed a lot of single neurons and surprisedly found whose axon branches were extremely numerous and complicated. The results indicated that this method can robustly and sparsely label stable number of cell-type specific neurons with super high brightness.

Disclosures: S. Jin: None. **X. Li:** None. **S. Tao:** None. **A. Li:** None. **F. Xu:** None. **H. Gong:** None.

Poster

251. Anatomic Methods: Circuit Tracing

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Program #/Poster #: 251.09/BB12

Topic: I.03. Anatomical Methods

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NIH 5RO1DA036913
Brain Research Foundation BRF-SIA-2014-03

IARPA D16PC0008
Simons Foundation 382793
Paul Allen Distinguished Investigator Award
Boehringer Ingelheim Fonds

Title: Bricseq bridges brain-wide interregional connectivity to neural activity and gene expression in single animals

Authors: *L. HUANG[1], J. M. KEBSCHULL[1], D. FURTH[1], S. MUSALL[1], M. T. KAUFMAN[2], A. K. CHURCHLAND[1], A. M. ZADOR[1];
[1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [2]Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL

Abstract: Comprehensive analysis of neuronal networks requires brain-wide measurement of connectivity, neural activity, and gene expression. Although high-throughput methods have been developed to map brain-wide neural activity and transcriptomes, comparable methods for generating region-to-region connectivity maps remain slow and expensive because they require pooling results across hundreds of brains. Here we describe BRICseq (BRain-wide Individual-animal Connectome sequencing), which leverages DNA barcoding and high-throughput sequencing to generate connectivity maps from single individuals in a few weeks and at low cost. Applying BRICseq to the mouse neocortex, we find that region-to-region connectivity provides a simple bridge for relating spatial patterns of gene expression to ongoing neuronal activity: the spatial expression patterns of a small number (~10) of genes predicts region-to-region connectivity, and connectivity in turn predicts region-to-region correlations in neural activity. We also exploited BRICseq to map the mutant BTBR mouse brain, which lacks a corpus callosum, and recapitulated its known connectopathies. BRICseq allows individual laboratories to compare how age, sex, environment, genetics and species affect neuronal wiring, and to integrate these with functional activity and gene expression.

Disclosures: L. Huang: None. **J.M. Kebschull:** None. **D. Furth:** None. **S. Musall:** None. **M.T. Kaufman:** None. **A.K. Churchland:** None. **A.M. Zador:** None.

Poster

251. Anatomic Methods: Circuit Tracing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 251.10/BB13

Topic: I.03. Anatomical Methods

Title: Quantification of nerve endings in mouse colorectum by optical tissue clearing and automatic image processing

Authors: *S. PATEL[1], N. GANESHBABU[1], T. GUO[2], M. HAN[2], B. FENG[2];
[1]Physiol. and Neurobio., [2]Biomed. Engin., Univ. of Connecticut, Storrs, CT

Abstract: Background: Irritable Bowel Syndrome (IBS) is a chronic gastrointestinal (GI) disorder that affects 10-20% of the American population, and prolonged visceral pain is the leading reason for IBS patients to visit GI clinics. IBS is a 'functional' GI disorder because apparent structural damage to the colon and rectum (colorectum) is lacking whereas the neural encoding functions of the peripheral afferents are enhanced, i.e., peripheral sensitization. We previously demonstrated long-term sensitization of stretch-sensitive colorectal afferents in three IBS-like mouse models with prolonged behavioral visceral hypersensitivity, i.e., mice receiving intracolonic treatments of trinitrobenzene sulfonic acid (TNBS), zymosan, and acidic hypertonic solution, respectively. Quantifying the morphology of these nerve fibers in both control and IBS mice will complement our neurophysiological understandings to provide insight into the alteration of nerve fiber distribution, shape, and their physical properties in disease states. This preliminary study highlights the accurate and high-throughput quantification of nerve ending morphologies through the thickness of mouse colorectal wall via optical tissue clearing and automatic nerve fiber detection with the Imaris software. Methods: We selectively labeled VGLUT2-positive neurons in mouse colorectum by crossing a VGLUT2-Cre strain with a fluorescent reporter strain. We conducted optical tissue clearing of the colorectum by adapting the SeeDB protocol originally developed for the brain, allowing confocal imaging of labeled nerve fibers in whole-mount colorectum. Automated and manual features of the Imaris software were used to quantify nerve fiber length, degree of curl, and distributions in different colorectal layers. Results: VGLUT2-Cre promoter labeled over 90% of neurons in the dorsal root ganglion, about 30% neurons in the myenteric plexus, and no neurons in the sympathetic or major pelvic ganglia. VGLUT2-positve nerve fibers are comparable in diameter throughout the colorectal wall, and are concentrated at the submucosal and myenteric plexus. Interestingly, fibers in the submucosa have significantly higher degree of curls than fibers in the myenteric plexus. Conclusion: The automatic and manual detection methods in Imaris are robust and efficient for precise quantification of the nerve fiber morphologies in optically cleared mouse colorectum. These accurate and high-throughput methods of nerve fiber quantification will allow sensitive detection of altered nerve fiber morphologies in IBS colorectum in future studies.

Disclosures: S. Patel: None. **N. Ganeshbabu:** None. **T. Guo:** None. **M. Han:** None. **B. Feng:** None.

Poster

251. Anatomic Methods: Circuit Tracing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 251.11/BB14

Topic: I.03. Anatomical Methods

Title: Neuron assembler: A Vaa3D submodule enables semi-automated neuron tracing with fragment units in real time

Authors: *H. KUO, Y. WANG, E. SHEN, A. FEINER, P. LESNAR, H. PENG;
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Abstract: Neuron morphology provides important clues to the study of cell types and functions. The reconstruction of neuron structures mostly relies on annotators' manual delineation. Although there have been attempts of automated neuron tracing tools with different methods, none of them has been proven to be successful in all situations and often need presumed conditions to be effective. Thus, reconstructing neurons remains a labor-intensive and time-consuming task for annotators.
The annotators' experience shows that correcting auto-traced neurons often results in even more time spent than tracing neurons entirely manually. Adjusting topological errors caused by false branching and false signal interpretation is the bottleneck that slows down the tracing process. In this work, we propose a semi-automated neuron tracing tool which aims to increase the annotator's tracing speed while avoiding as many topological errors as possible. This goal is achieved by representing neuron fibers with simple fragments without forming a complex topology for annotators. Therefore, the annotators' manual work is reduced to connecting fragments and essential line-drawing for higher level structures.
In the terafly environment of Vaa3D, an image block is first enhanced and segmented to generate a binary image mask. The string-like nature of neuron fibers indicates that the skeletons of segmented objects are essentially the fragments to be used to build up the neuron structure. To allow instant interaction between annotators and the computer, we re-designed the 3D connected component and 3D skeletonization algorithms to produce neuron fragments in real time. Finally, all branches are broken, and small fragments are eliminated with a user-defined threshold. The module also provides convenient tools to help annotators label, track, and connect neuron segments. Our testing results show that Neuron Assembler can help annotators maneuver even in a dense region. In 3 types of local mouse brain regions (dense dendritic, dense axonal, and bundled axon), we asked 4 annotators to time the tracing with and without neuron fragments provided by Neuron Assembler. Compared with tracing entirely manually, on average Neuron Assembler reduced 24.13%, 11.67%, and 27.97% of the time needed to complete dense dendritic, dense axonal, and bundled axon regions, respectively. It is shown that an experienced annotator can finish tracing 1 neuron within 1 day from a sparsely labeled whole mouse brain with Neuron Assembler, whereas all-manual tracing process would normally take several days. Neuron Assembler has now been incorporated into the Allen Institute's neuron tracing protocol for fMOST image data.

Disclosures: **H. Kuo:** None. **Y. Wang:** None. **E. Shen:** None. **A. Feiner:** None. **P. Lesnar:** None. **H. Peng:** None.

Poster

251. Anatomic Methods: Circuit Tracing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 251.12/BB15

Topic: I.03. Anatomical Methods

Support: NIH Grant EY028148
That Man May See Foundation
NVIDIA GPU Grant
Reseatch to Prevent Blindness
NIH Grant EY002162

Title: Automatic large-scale, deep learning-assisted synapse recognition in the central nervous system

Authors: *L. DELLA SANTINA, A. K. YU, Y. OU;
Dept. of Ophthalmology, Univ. of California, San Francisco, San Francisco, CA

Abstract: The central nervous system contains trillions of synapses. Our ability to quantify them has always been limited by technical constraints such as imaging resolution and area, as well by our ability to manually detect synapses from noise in large image stacks, thus precluding the possibility of exploring in full extent the general rules behind developmental and degenerative processes.
We developed a novel open source software suite able to automatize the quantification of synapses in large three-dimensional image datasets obtained with confocal or super-resolution microscopes. In addition, our software is able to perform colocalization analysis and Monte Carlo simulations of synapse development, rearrangement, and degeneration. We trained and employed a convolutional deep-learning neural network to perform automatic validation of synapses with nearly real-time speed and tested it in the mouse retina as a model for central nervous system synapses. This approach revealed that in a mouse model of glaucoma, contrary to previous assumptions, retinal ganglion cell synapse disassembly initiates from the presynaptic side, following sublamina-specific spatial distribution and cell type-specific temporal order. Automatic recognition and validation of synaptic protein labeling in immunofluorescence images represents an efficient and objective approach to explore large volumes of neural tissue, enabling the investigator to interrogate synaptic properties of complete regions of the CNS, thus revealing regional details in spatial synaptic distribution that were previously unappreciated.

Disclosures: L. Della Santina: None. **A.K. Yu:** None. **Y. Ou:** None.

Poster

251. Anatomic Methods: Circuit Tracing

Location: Hall A

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Program #/Poster #: 251.13/BB16

Topic: I.03. Anatomical Methods

Support: NIH Grant U01 MH11699001
NIH Grant R01 DC00898312

Title: Anterograde transsynaptic AAV techniques for probing neural circuitry

Authors: *B. ZINGG, B. PENG, H. TAO, L. ZHANG;
USC, Los Angeles, CA

Abstract: Revealing the organization and function of neural circuits is greatly facilitated by viral tools that spread transsynaptically. Adeno-associated virus (AAV) has recently been shown to be capable of anterograde transneuronal transport, with serotype 1 in particular exhibiting the greatest efficiency of spread. The extent to which AAV1 spreads specifically across synaptic connections, however, remains uncertain. We therefore systematically examined the synaptic specificity of AAV1 transneuronal transport using a variety of functional and anatomical approaches. First, we found a strong correspondence between pre-synaptic connectivity and post-synaptic labeling in slice recording experiments. Then, in anatomical tests of synaptic specificity, we found that AAV1 selectively labels expected cerebellar granule or Purkinje cell populations downstream of pontine or olivary projection pathways, respectively. In addition, we established that AAV1 is transported efficiently through inhibitory projection pathways and long-range corticospinal pathways, but shows little or no spread through neuromodulatory projections (e.g. serotonergic, cholinergic, and noradrenergic). Lastly, we incorporated its use with approaches for achieving sparse labeling of input- and genetically-defined neurons to facilitate their morphological reconstruction. Together our results suggest that AAV1 spreads to downstream neurons with a high degree of synaptic specificity, may be used in a wide variety of projection pathways, and shows great potential for further optimization as an anterograde transsynaptic tool.

Disclosures: **B. Zingg:** None. **B. Peng:** None. **H. Tao:** None. **L. Zhang:** None.

Poster

251. Anatomic Methods: Circuit Tracing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 251.14/BB17

Topic: I.03. Anatomical Methods

Title: High resolution MRI/DTI and tractography study of the postmortem human basilar pons

Authors: *C. SKLIROS[1], K. W. ASHWELL[2], A. JOHNSON[3], E. CALABRESE[3], M. AGGARWAL[4], G. PAXINOS[5];
[1]Anat., NeuRA - UNSW, Sydney, Australia; [2]Anat., UNSW, Sydney, Australia; [3]Dept. of Radiology, Ctr. for In Vivo Microscopy, Durham, NC; [4]Dept. of Radiology and Radiological Sci., Russel H. Morgan Dept. of Radiology, Baltimore, MD; [5]Neura - UNSW, Sydney, Australia

Abstract: Aims: The aim of this study is to map the course and the components of the crus cerebri and the longitudinal fibres of the pons using ultra-high resolution magnetic resonance imaging. **Methods:** A postmortem human brain was obtained from a 65-year-old male. MR imaging was performed in a modified 7T MRI system controlled with an Agilent console, rendering a 50-µm resolution gradient recalled echo (GRE) and a 200-µm resolution directionally coloured fractional anisotropy (FAC). Tractography was performed using DSI Studio. A HARDI scheme was used, and a total of 120 diffusion sampling directions were acquired. **Results:** This study revealed that the external part of the internal capsule is occupied by the fibres that will eventually constitute the cervical pyramidal tract, the intermediate part by those of the thoracic, and the internal part by those of the lumbar and sacral. A portion of the fibres from the internal part of the internal capsule and crus cerebri are distributed to the substantia nigra pars reticulata. The remaining part of the crus cerebri together with its internal, intermediate and external parts constitute the longitudinal fibres of the pons with the corticospinal fibres continuing to the cervical, thoracic, lumbar and sacral cord. Caudal to the internal capsule the descending fibres shift from an external to a ventrolateral position in the crus cerebri where they divide into 9 distinct fibre bundles (with a degree of interchanges). As they pass through the mid pons they converge and from their dorsoventral orientation they shift medially and form the cervical, thoracic, lumbar and sacral parts of the pyramidal tract. **Conclusion:** By high resolution MRI/DTI, the course of the components of the crus cerebri and the longitudinal fibres of the pons have been demonstrated with higher accuracy than before. Investigation revealed that the descending fibres are not haphazardly arranged, but maintain organisation of topography, and travel in distinct bundles through the basilar pons.

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Poster

251. Anatomic Methods: Circuit Tracing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 251.15/BB18

Topic: I.03. Anatomical Methods

Support: FRIPRO ToppForsk grant Enhanced Transgenics (90096000)

Title: Brain-wide quantitation of monosynaptic inputs to subdivisions of the claustral complex

Authors: *J. S. GRIMSTVEDT[1], C. H. BERNDTSSON[2], S. BLANKVOORT[1], R. R. NAIR[3], M. P. WITTER[1], C. G. KENTROS[1];
[1]Kavli Inst. Systems Neuroscience, Norw. Univ. Sci. & Tech., Trondheim, Norway; [2]MH Fac. Admin., Trondheim, Norway; [3]The Kavli Inst. For Systems Neurosci. / CNC, Trondheim, Norway

Abstract: Much can be inferred about the function of a brain region by investigating its connectivity. This is especially true for the claustral complex (CC), a deep lying gray matter region in the forebrain composed of the claustrum (CL) and the dorsal endopiriform nucleus (DEn). Several theories exist regarding the function of the CC, ranging from selective attention to consciousness, but the commonality of all these theories is that they are largely based upon connectivity. However, the CC is notoriously difficult to target stereotactically, and as a result there have been few brain-wide investigations of its connectivity. Additionally, most research on the CC in rodents targeted the CL, whereas the DEn remains largely unexplored. Our goal was to create a more complete picture of the brain-wide input connectivity of the CC, and to identify input variation between its dorsoventral and rostrocaudal subdivisions. By using a transgenic mouse line that allows region specific targeting of the CC, we were able to retrogradely trace its monosynaptic inputs with glycoprotein gene-deleted rabies virus. Input cells were found in a plethora of cortical and subcortical regions, with prominent contributions from prefrontal cortices, hippocampus, parahippocampus, amygdala and olfactory areas. Using a regression-based analysis we were able to identify linear relationships between input regions and subdivisions of the CC. This allowed us to characterise differences between the connectivity of the CL and the DEn, both at rostral and caudal levels. Our findings corroborate and expand previous knowledge regarding claustral connectivity, and demonstrate variations in input connectivity between subdivisions of the CC. Interestingly, several input regions in the hippocampus and parahippocampus were correlated to caudal parts of the CC, warranting further investigation of this subdivision in memory processing.

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Poster

251. Anatomic Methods: Circuit Tracing

Location: Hall A

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Program #/Poster #: 251.16/BB19

Topic: I.03. Anatomical Methods

Support: NIH Grant R01AA024774
NIH-NIGMS5R25GM099649-03

Title: Optimizing techniques to visualize and quantify alcohol-induced changes to myelinated axons of the medial prefrontal cortex

Authors: *A. SILVA-GOTAY[1], K. LUCIER[1], H. N. RICHARDSON[2];
[1]Psychological and Brain Sci., [2]Dept. of Psychological and Brain Sci., Univ. of Massachusetts Amherst, Amherst, MA

Abstract: Adolescence is a time of plasticity when neural circuits undergo maturational changes that impact neural processing and behavior. In humans, binge drinking at an early age is associated with cognitive impairments and alcohol use disorder later in adulthood. In line with these findings, we found that early adolescent drinking reduces myelin in the medial prefrontal cortex (mPFC). Identification of the specific axonal circuit affected by alcohol is necessary to better understand the underlying mechanisms and functional consequences of these axonal changes. The current project focused on optimizing techniques to distinguish between myelinated and unmyelinated axons within prefrontal pathways with the long-term goal of isolating the specific prefrontal axons impacted by alcohol. We first compared different tract tracers in male and female rats and found that intra-mPFC injection of 3,000 MW fluorescein dextran amine (FDA) resulted in superior axonal labeling compared to adeno-associated virus (AAV2/1-CAG-GFP). We then confirmed that FDA targets both anterograde and retrograde transport along mPFC axons, and we determined that we could manipulate the survival time to optimize the labeling of longer projecting fibers. Brain tissue slices were next processed for either immunofluorescent labeling of myelin or label-free imaging of myelin using spectral confocal reflectance (SCoRe) microscopy. We found that SCoRe can be performed in 35 um tissue sections and that this approach was better option for visualizing myelin on traced axons when compared to immunolabeling. Finally, we assessed quantification approaches and established that the density of afferent and efferent myelinated axons can be quantified by combining colocalization and threshold analysis of SCoRe using NIS Elements Advanced Research analysis software. This combination of techniques can be used to assess changes in specific myelinated fiber pathways under baseline conditions and after adolescent alcohol.

Disclosures: A. Silva-Gotay: None. **K. Lucier:** None. **H.N. Richardson:** None.

Poster

251. Anatomic Methods: Circuit Tracing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 251.17/BB20

Topic: I.03. Anatomical Methods

Support: 1U01MH114829-01

Title: Anatomical characterization of neuronal cell types of the mouse brain

Authors: *H.-W. DONG[1], G. A. ASCOLI[2], B. LIM[3], I. R. WICKERSHAM[4], H. HINTIRYAN[5];
[1]Keck Sch. of Med. of USC, Los Angeles, CA; [2]George Mason Univ., Fairfax, VA; [3]Biol. Sci., UCSD, La Jolla, CA; [4]McGovern Inst. for Brain Res., MIT, Cambridge, MA; [5]USC, Los Angeles, CA

Abstract: A comprehensive understanding of neuronal cell type diversity will provide an essential guide to their selective manipulation and illuminate cell type specific contributions to health and disease. As part of the Brain Initiative Cell Census Network (BICCN), our project aims to fully characterize the anatomic features of neuronal cell types. Collectively our experiments will reveal cell type anatomic location, morphology, and comprehensive connectivity across different resolutions and their monosynaptic input/output organization. To this end, we have been making progress in the following aspects of the proposed project: (1) Producing mesoscale quadruple retrograde tracing data, mostly focusing on the primary motor cortex and hippocampus in Year 1. These data will initially characterize cell types based on the anatomical location of their connectional start and end points. A shot-gun approach is developing to ensure systematic and comprehensive characterization of projection neuron types. (2) Applying advanced viral tracing tools to subsequently refine specific axonal projections, collaterals, and projection fields of the cell types. Combinatorial tracing methodologies are applied to determine the discrete connectional inputs providing a more complete anatomical characterization of the different cell populations; (3) Developing brain clearing (CLARITY, SHIELD) and 3D imaging technologies to reveal the morphological features of rabies labeled neurons. In a combination of advanced viral tracing, SHIELD, and novel brain expansion technology, this approach can be applied to determine the spatial organization of synaptic inputs from different sources to their corresponding cell types. (4) Developing informatics tools for high-throughput reconstruction of neurons (i.e., a new computational tool called G-Cut) and online repositories of neuronal morphologies and of cell types (i.e., NeuroMorpho.Org and Hippocampome.org). We will develop a web-based visualization platform that enables users to view, analyze, and compare all cell type anatomy data produced in the project. In the meeting, we will present an update of our progress.

Disclosures: **H. Dong:** None. **G.A. Ascoli:** None. **B. Lim:** None. **I.R. Wickersham:** None. **H. Hintiryan:** None.

Poster

251. Anatomic Methods: Circuit Tracing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 251.18/BB21

Topic: I.03. Anatomical Methods

Support: NIH Grant AG047589

Title: Unsupervised construction of a data-driven cortical hierarchy in mouse

Authors: H. CHOI[1], K. E. HIROKAWA[2], J. D. WHITESELL[2], N. GRADDIS[2], C. KOCH[2], H. ZENG[2], *J. A. HARRIS[2], S. MIHALAS[2];
[1]Applied Mathematics, Univ. of Washington, Seattle, WA; [2]Allen Inst. for Brain Sci., Seattle, WA

Abstract: The mammalian cortex is a laminar structure composed of many areas and cell types densely interconnected in complex ways. The organization of inter-areal connectivity has been studied using graph theory (Bullmore, & Sporns 2009). However standard graph theory generally does not distinguish types of connections or cell types. Extensions of this work, in terms of multigraphs, are important given the diversity of cell types and connection types observed. One of the simpler extensions, the concept of a cortical hierarchy (Felleman & Van Essen, 1991) has been useful for understanding computational and architectural properties of the cortex, and has inspired the development of neural network methods in machine vision (Riesenhuber & Poggio, 1999). Here we describe and apply an algorithm to automatically discover a hierarchy in the mouse cortex. We use a large-scale dataset on cell class-specific connectivity between cortical areas defined using Cre driver transgenic lines (Harris et al. 2018). First, for each measured transgenic line, we quantify the strength and layer termination pattern of all the inter-areal cortical connections. For the connections above a threshold we perform an unsupervised clustering of the layer termination patterns. While simultaneously assigning a hierarchical position to each cortical area, we attach directionality (feedforward or feedback) to each connection. We search over all possible mappings between hierarchical directions and cluster membership, to find the most self-consistent hierarchical structure. The observed mapping between clusters and hierarchical directions is consistent with observations from trained anatomists, also showing how fantastic the human brain is at pattern recognition. The observed hierarchy also matches expectations based on previous literature. We extend this work to include thalamo-cortical and cortico-thalamic connections using the same general principles of searching for a self-consistent hierarchy. Our results suggest that connections across the entire mouse

cortex and thalamus are hierarchically organized in a way that defines pathways and directions of information flow across the cortical thalamic network.

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Poster

251. Anatomic Methods: Circuit Tracing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 251.19/DP14/BB22

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: I.03. Anatomical Methods

Support: NIH RF1MH117815
IARPA DoI/IBC D16PC0005

Title: Crowdsourcing the connectome of the female adult fly brain

Authors: C. E. MCKELLAR[1], K. LEE[1], N. L. TURNER[1], S.-C. YU[1], R. LU[1], J. WU[1], Z. JIA[1], E. MITCHELL[1], B. NEHORAN[1], S. POPOVYCH[1], M. CASTRO[1], A. HALAGERI[1], C. JORDAN[1], N. KEMNITZ[1], W. SILVERSMITH[1], J. ZUNG[1], F. COLLMAN[3], S. DORKENWALD[1], *T. MACRINA[1], K. LI[2], **M. MURTHY**[1], H. SEUNG[1];
[1]Princeton Neurosci. Inst., [2]Computer Sci., Princeton Univ., Princeton, NJ; [3]Allen Inst. for Brain Sci., Seattle, WA

Abstract: Wiring diagrams at single-synapse resolution are highly-desirable for neural circuit analyses. We present a high-quality automated segmentation and synapse detection, based on a serial section electron microscopy dataset (Zheng et al. 2018), and a proofreading platform by which we are reconstructing circuits in the *Drosophila melanogaster* female adult brain. Our platform is open to the community to collaboratively improve the reconstruction toward this individual's complete connectome.Zheng, Zhihao, J. Scott Lauritzen, Eric Perlman, Camenzind G. Robinson, Matthew Nichols, Daniel Milkie, Omar Torrens, et al. 2018. "A Complete Electron Microscopy Volume of the Brain of Adult *Drosophila Melanogaster*." Cell 174 (3): 730-43.e22.

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Poster

251. Anatomic Methods: Circuit Tracing

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Program #/Poster #: 251.20/DP15/BB23

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: I.03. Anatomical Methods

Support: HHMI

Title: The Janelia MouseLight Project: A platform for brain-wide reconstructions and molecular characterization of individual neurons in the mouse brain

Authors: *T. A. FERREIRA, J. WINNUBST, E. BAS, A. RECKNAGEL, J. DUDMAN, C. GERFEN, A. HANTMAN, W. KORFF, S. MURPHY, N. SPRUSTON, S. STERNSON, K. SVOBODA, J. CHANDRASHEKAR;
HHMI - Janelia Res. Campus, Ashburn, VA

Abstract: The structure and fine-scale axonal arborizations of neurons dictate how information from individual neurons is routed across the brain. In addition, neuronal morphology is a rich descriptor of cell type. Despite such a fundamental role for structure, few mammalian projection neurons have been reconstructed in their entirety. The Janelia MouseLight project has assembled a robust and efficient pipeline for imaging and reconstructing complete neuronal morphologies of single neurons in the mouse brain. All resources necessary for implementing this pipeline—hardware designs and custom software—are open-source. We have combined this workflow with *post-hoc* multiplexed smFISH for molecular profiling of reconstructed cells. Using this platform, we have reconstructed complete morphologies of more than 1,500 neurons from key areas of the brain, including motor cortex, thalamus, hypothalamus, and subiculum. Analysis of this dataset has revealed previously unknown subtypes of projection neurons; in some cases, these new projection types correspond to specific gene expression patterns determined using single-cell RNA sequencing (e.g. BICCN). All reconstructed neurons are shared via an online database with extensive search and visualization capabilities (mouselight.janelia.org). Making these data available to the broader community will help translate neuronal structure to novel insights about the development, function, and dysfunction of the mammalian brain.

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Poster

251. Anatomic Methods: Circuit Tracing

Location: Hall A

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Program #/Poster #: 251.21/BB24

Topic: I.03. Anatomical Methods

Support: R01 MH094360-01A1
1RF1MH114112-01
U01MH114829

Title: Mouse connectome project (MCP): Towards the construction of the whole brain wiring diagram

Authors: *H. HINTIRYAN, M. BIENKOWSKI, N. N. FOSTER, I. BOWMAN, L. GOU, M. BECERRA, M. ZHU, M. Y. SONG, N. L. BENAVIDEZ, L. GAO, K. COTTER, S. YAMASHITA, C. CAO, S. AQUINO, N. KHANJANI, D. LO, D. L. JOHNSON, G. DAN, T. BOESEN, S. USTRELL, M. FAYZULLINA, L. GARCIA, L. KOROBKOVA, S. WANG, H. XU, B. ZHANG, H.-W. DONG;
USC, Los Angeles, CA

Abstract: The long term objective of our Mouse Connectome Project (MCP, www.MouseConnectome.org) is to map the interconnections among all regions of the C57Bl/6 mouse brain, to generate a corresponding comprehensive connectome map that represents the interconnections in a common neuroanatomic frame, and to understand how the different brain regions assemble into functional networks based on these connections. As a part of the NIH BRAIN Initiative (https://www.braininitiative.nih.gov/), our MCP has made significant progress over the last 7 years. We have generated the first and the most comprehensive connectomic map of the cerebral cortex available for any mammalian species (Zingg et al., Cell, 2014). Computational analysis of this map revealed that the mammalian cerebral cortex, long thought to be a dense single interrelated tangle of neural networks, was composed of relatively few functionally segregated cortico-cortical subnetworks. Subsequently, we also constructed (1) a comprehensive mesoscale mouse cortico-striatal projectome (Hintiryan et al., Nature Neuroscience, 2016), which is a detailed connectivity projection map from the entire cerebral cortex to the dorsal striatum or caudoputamen in rodents; (2) the genetic architecture and wiring diagram of the mouse hippocampus (Bienkowski et al., Nature Neuroscience, 2018). Following the same principle and strategy, we project to systematically and comprehensively assemble the global neural networks and a Google earth-like map of the entire mouse brain within the next 5 years. As part of the effort to accelerate scientific advancements through open resources and data sharing, our high-resolution, high quality raw images of connectivity data are presented through a publicly accessible database—the iConnectome. The graphic reconstructions of the

connections summarized in connectivity maps are also available through iConnectome maps. Our future direction includes constructing these global networks at the cellular level and the further customization of our existing informatics tools to facilitate data visualization and analysis (Sponsored by R01 MH094360-01A1, 1RF1MH114112-01, U01MH114829).

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Poster

251. Anatomic Methods: Circuit Tracing

Location: Hall A

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Program #/Poster #: 251.22/BB25

Topic: I.03. Anatomical Methods

Support: MnDrive Brain Conditions
NARSAD Young Investigator Award
NIH R01MH118257

Title: Cross-species circuit identification of components of the posteromedial cortex

Authors: *M. E. MONKO, L. TOKA, P. KANDIKONDA, A. SETHI, S. R. HEILBRONNER; Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: The posteromedial cortex (PMC) is the posterior hub of the default mode network, which has been implicated in a wide range of functioning including rest state and autobiographical thought. In humans and nonhuman primates, the PMC consists of the posterior cingulate cortex, the precuneus, and the retrosplenial cortex. Although studies have confirmed the existence of a default mode network in humans, nonhuman primates, and rodents, we do not know the extent to which the PMC, as a region, in rodents is similar to what is seen in humans and nonhuman primates. Knowing homology of this region will benefit the field, as rodents are often used as models for psychiatric and neurological diseases.To this aim, we used tract-tracing techniques to provide a circuit-based homological identification of the PMC in rats and macaques. Using bidirectional tracers, we examined the organization projections to and from the PMC in adult rats and nonhuman primates (rhesus macaques). Resulting labeled cells and terminal fields were examined and charted under a Zeiss AxioImager with StereoInvestigator and NeuroLucida technologies. We particularly focused on anatomical connectivity with conserved structures within the striatum, prefrontal cortex, and medial temporal lobes. For each PMC case,

we made a 3D model of the connections, then registered these to an MRI-generated reference space. Our results indicate broad homology in anatomical connectivity between the rodent and nonhuman primate PMC, and, although preliminary in nature, may suggest that portions of the rodent PMC are similar not just to the retrosplenial cortex, but also the posterior cingulate cortex. Implications for default mode organization will be discussed.

Disclosures: M.E. Monko: None. **L. Toka:** None. **P. Kandikonda:** None. **A. Sethi:** None. **S.R. Heilbronner:** None.

Poster

251. Anatomic Methods: Circuit Tracing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 251.23/BB26

Topic: I.03. Anatomical Methods

Support: UMN Medical Discovery Team on Addiction Pilot Grant
MnDrive Brain Conditions
NARSAD Young Investigator Award
R01MH118257

Title: Use of viral tools in the analysis of neuronal circuits in nonhuman primates

Authors: *A. CUSHNIE[1], M. WANG[1], E. MARRON[2], M. MONKO[1], M. GRIER[1], T. CASTA[1], S. HEILBRONNER[1];
[1]Neurosci., [2]Pharmacol., Univ. of Minnesota, Minneapolis, MN

Abstract: Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), synthetic variants of G-Protein Coupled Receptors (GPCRs), can be selectively targeted to specific neuronal circuits. DREADDs allow for transient remote manipulation of specific neuronal circuits in freely moving animals and are pharmacologically activated when bound by exogenous compounds, administered systemically or by local infusions. DREADDs are widely used in rodent models, however limited progress has been made to virally target and manipulate neuronal circuits in nonhuman primates (NHPs). To this end, we performed stereotaxically-guided viral vector delivery of DREADDs to cortical and striatal subregions of *Macaca mulatta.* We used the following constructs across multiple individuals: *AAV8-CaMK11-hM4Di-GFP, AAV8-CaMK11-hM4Di-mCherry, AAV5-hSyn-hM4Di-HA, AAV5-CaMK11-hM4Di-GFP AAV5-CaMK11-hM4Di-mCherry, rAAV2retro-hSyn-hM4Di-HA, rAAV2retro-CaMKII-hM4Di-GFP, rAAV2retro-CaMKII-hM4Di-mCherry*. We also varied the volume and method of delivery. Brains were perfused, extracted, histologically stained, and analyzed under light and fluorescence microscopy. Our goal is to determine the optimal viral construct for DREADDs delivery in different experimental circumstances. To this end, we investigate the transduction

rate, transport efficacy (relative to similar cases using traditional tract tracers), and toxicity. We also report on systemic immune responses and strategies for prevention. These proof of concept studies reveal the underlying anatomical connectivity and set the stage for more detailed network interrogation and behavioral experiments using DREADDs technology.

Disclosures: M. Wang: None. **E. Marron:** None. **M. Monko:** None. **M. Grier:** None. **T. Casta:** None. **S. Heilbronner:** None. **A. Cushnie:** None.

Poster

251. Anatomic Methods: Circuit Tracing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 251.24/BB27

Topic: I.03. Anatomical Methods

Title: Functional annotation of single-neuron whole-brain axonal projections in the cerebral cortex in mice

Authors: *W. ZHOU, Y. CAO, J. CHANG, H. GONG;
Britton Chance Ctr. for Biomed. Photonics, Wuhan Natl. Lab. for Optoelectronics-Huazhong Univ. of Sci. and Technol., Wuhan, China

Abstract: At present, there are still some gaps in the research between the structure and function of neurons. Structural studies provide morphological information of neurons, which indicates the physiological status, dendrites shape and axonal projections. In particular, whole-brain imaging techniques developed in recent years have not only revealed the fine structure of neurons, but also found that the axons of most cortical neurons have whole-brain long-range projection. Meanwhile, functional studies have found that the neurons in cerebral cortex, as a relay station for sensory, motor and other informations, presents diverse functions. However, it is difficult to combine long-range projection with precise function. Among existing technologies, at the level of single neuron, fluorescent Micro-Optical Sectioning Tomography (fMOST) technique can collect whole-brain projection information of single neuron, while two-photon imaging can obtain functional characteristics of single neuron. Here, we established a method to combine two-photon imaging with fMOST technique to obtain the functional information of single neuron and whole-brain projection, so as to provide functional annotation for cortical single-neuron projection. By further studying the correlation between neuronal function and projection characteristics, the projection rule of cortical neurons was revealed. This work will provide more details for the discovery of neural projection rule and new ideas on the construction rule of neural circuit.

Disclosures: W. Zhou: None. **Y. Cao:** None. **J. Chang:** None. **H. Gong:** None.

Poster

251. Anatomic Methods: Circuit Tracing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 251.25/BB28

Topic: I.03. Anatomical Methods

Title: Whole brain map of long-range input of different interneurons in CFA

Authors: *X. LI[1], Z. DUAN[2];
[1]Wuhan Natl. Lab. For Optoelectronics, Wuhan, China; [2]Huazhong Univ. of Sci. and Technol., Wuhan, China

Abstract: The inhibitory regulation in forelimb movement is mainly driven by caudal forelimb area in neocortex (CFA), which provide long-range GABAergic projections to the striatum and in local. To generate brain-wide maps of the input to interneuron in two sub-regions of CFA, we used the monosynaptic rabies virus system, combine with mice expressing Cre recombinase in either parvalbumin-positive (PV), somatostatin-positive (SST), or vasoactive intestinal peptide-positive (VIP) neurons, to analyze the major input regions in whole brain level quantitatively. Despite the similar input sources of these interneurons, we found that different sub-regions of CFA receive long-range inputs in remarkable different distribution and proportions from cortex and thalamus. The inputs from SSp-bfd and SSs were anatomically organized in two cortical circuits. The segregation of input distribution in posterior medial thalamus from ambilateral region to central region supported the concept of parallel pathway in the motor system. The map give insight into the inhibitory regulate processes in forelimb movement and the structural architecture underlying the functions of CFA.

Disclosures: X. Li: None. **Z. Duan:** None.

Poster

251. Anatomic Methods: Circuit Tracing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 251.26/BB29

Topic: I.03. Anatomical Methods

Support: NIH Grant NS091421

Title: NCTracer web application for registration, visualization, and neuron tracing in large-scale light microscopy datasets

Authors: *S. MOUSAVI KAHAKI, A. VYAS, C. LIU, R. LI, S.-L. WANG, M. RIEDEWALD, A. B. STEPANYANTS;
Northeastern Univ., Boston, MA

Abstract: The ability to map synaptic connectivity of neural networks at single-cell resolution is critical for advancing the understanding of basic brain functions. Due to the long-range nature of connectivity, comprehensive circuit mapping must be done on the scale of an entire brain with automated, high-throughput technology. It is now possible to fluorescently label distinct populations of neurons and image their axonal and dendritic arbors in 3D from multiple optical sections. Reconstruction of neurites embedded in such images is the limiting factor in large-scale circuit mapping projects. Here, we present *NCTracer Web*, a web-based software for visualization and online collaborative tracing of neurites in terabyte-size imaging datasets. At present, functionalities of *NCTracer Web* include registration of microscopy images, image filtering, visualization and navigation through the 3D space of images, and multi-user manual tracing. Automated tracing and computer-guided trace proofreading functionalities are currently under development. The application core of *NCTracer Web* consists of a database published via web services and a distributed computation backend for massively parallel image processing executed on a computer cluster or in the cloud. The database indexes image volumes and traces of neurites for fast streaming to users' browsers. Trace data is synchronized centrally to enforce a consistent view to concurrent users. This architecture enables real-time collaborative tracing during which users can see the work of their remote colleagues. The careful distribution of functionality and data within *NCTracer Web* provides a reliable, performant, and scalable research tool.

Disclosures: S. Mousavi Kahaki: None. **A. Vyas:** None. **C. Liu:** None. **R. Li:** None. **S. Wang:** None. **M. Riedewald:** None. **A.B. Stepanyants:** None.

Poster

251. Anatomic Methods: Circuit Tracing

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 251.27/BB30

Topic: I.03. Anatomical Methods

Support: NIH Grant NS091421

Title: Registrar software for spatial and time-lapse registration of light microscopy images

Authors: S. MOUSAVI KAHAKI, C. LIU, A. VYAS, S.-L. WANG, R. LI, M. RIEDEWALD, ***A. B. STEPANYANTS**;
Northeastern Univ., Boston, MA

Abstract: The ability to map neural circuits on the scale of an entire brain and to monitor changes in the circuits over long periods of time is critical for advancing our understanding of brain functions. Circuit mapping and plasticity experiments typically involve imaging of sparsely labeled populations of neurons with 3D confocal or two-photon microscopy, often producing tens of thousands of stacks of images, totaling several terabytes of data per brain. Registration of images within individual stacks, registration of image stacks across space, and registration of image stacks over time are desired for seamless visualization and are required for accurate, automated analyses of the imaged data. Here, we propose a feature-based registration algorithm designed to address all three registration problems within a single framework. The algorithm first detects local maximum intensity regions in the stacks, and for every overlapping or consecutive stack pair establishes an initial correspondence between these features with the Hungarian algorithm. A random sampling-based refinement method is then employed to eliminate outliers, and the resulting sets of corresponding features in all stack pairs are used to find the optimal registering transformations for the stacks. Finally, the transformed stacks are retiled and blended into a set of non-overlapping images that can be used for visualization and analyses. Our algorithms, implemented in *Registrar* software, provide a utility for registration based on translation, rigid, affine, and B-spline transformations. The accuracy of *Registrar* was tested on several datasets representing distinct imaging modalities, systems, and experiments. The results show that *Registrar* consistently attains a sub-micrometer registration accuracy and compares favorably to the available registration tools.

Disclosures: S. Mousavi Kahaki: None. **C. Liu:** None. **A. Vyas:** None. **S. Wang:** None. **R. Li:** None. **M. Riedewald:** None. **A.B. Stepanyants:** None.

Poster

252. Connectomics Analytics III

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 252.01/BB31

Topic: I.07. Data Analysis and Statistics

Support: NIH R42CA173976

Title: Comparison of low-frequency resting-state fMRI and breath-hold metrics of cerebrovascular reactivity

Authors: ***N. J. FESHARAKI**[1,3], W. E. HUDDLESTON[4], J. REUSS[5], J. PILLAI[6], E. A. DEYOE[2];

[2]Dept. of Radiology, [1]Med. Col. of Wisconsin, Milwaukee, WI; [3]Univ. of Wisconsin-Milwaukee, Milwaukee, WI; [4]Kinesiology: Integrative Hlth. Care & Performance, Univ. of Wisconsin - Milwaukee, Milwaukee, WI; [5]Prism Clin. Imaging, Inc., Milwaukee, WI; [6]Dept. of Neurosurg., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: The neurovascular coupling mechanism responsible for fMRI signals can be focally disrupted by various pathological factors (such as brain tumors) while leaving the underlying neurons functionally intact. Such neurovascular uncoupling (NVU), can pose serious complications for clinical use of fMRI. To assess potential biomarkers for NVU, we tested two metrics of cerebrovascular reactivity (CVR) based on active breath-hold (BH) and passive resting-state (RS) fMRI paradigms. For 5 healthy subjects, we obtained a BH CVR metric by cross-correlating each subject's personalized respiratory response function with the fMRI time series of each voxel. The metric for RS was the fMRI signal power within a frequency band from 0.01 - 0.08 Hz. We then applied a predictive value analysis over a wide range of threshold settings to assess the accuracy of spatial correspondence between the two CVR metrics. The overall accuracy of correspondence was fairly modest, ranging from 0.60 to 0.68 (mean: 0.63). As a control, an identical analysis for two independent samples of RS CVR data revealed significantly higher accuracy compared to BH CVR data (0.80 vs 0.68, paired-sample t-test, $p < 0.05$). We also assessed the spatial overlap of each CVR metric with cortical gray matter. Correspondence accuracy was higher for the RS metric of CVR than the BH metric (0.67 vs 0.59, paired-sample t-test, $p < 0.05$). Our quantitative analysis suggests that the BH and RS fMRI maps of CVR are similar, but not identical at a voxel level of resolution. Moreover, their mutual correspondence and association with gray matter are critically dependent on the threshold setting used to identify active versus inactive vascular responses. The RS CVR metric segregates more accurately and has more complete coverage of gray matter than the BH metric. Additionally, the higher correspondence between two independent samples of the RS metric compared to two samples of the BH metric suggests that the RS CVR metric may be a modestly more reliable biomarker while also avoiding the need for consistent task performance of potentially compromised clinical patients.

Disclosures: N. J. Fesharaki: None. **W.E. Huddleston:** None. **J. Reuss:** None. **J. Pillai:** None. **E.A. DeYoe:** None.

Poster

252. Connectomics Analytics III

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 252.02/BB32

Topic: I.07. Data Analysis and Statistics

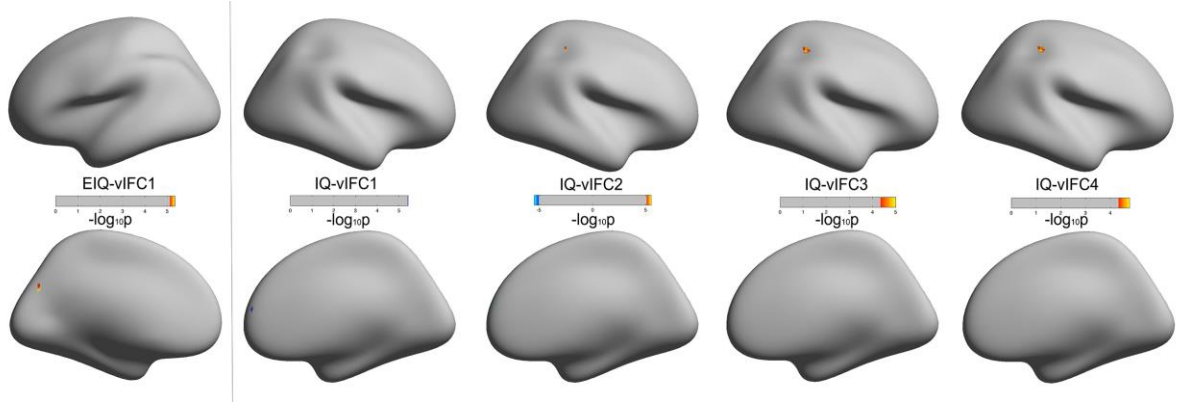
Support: NSFC 11674388

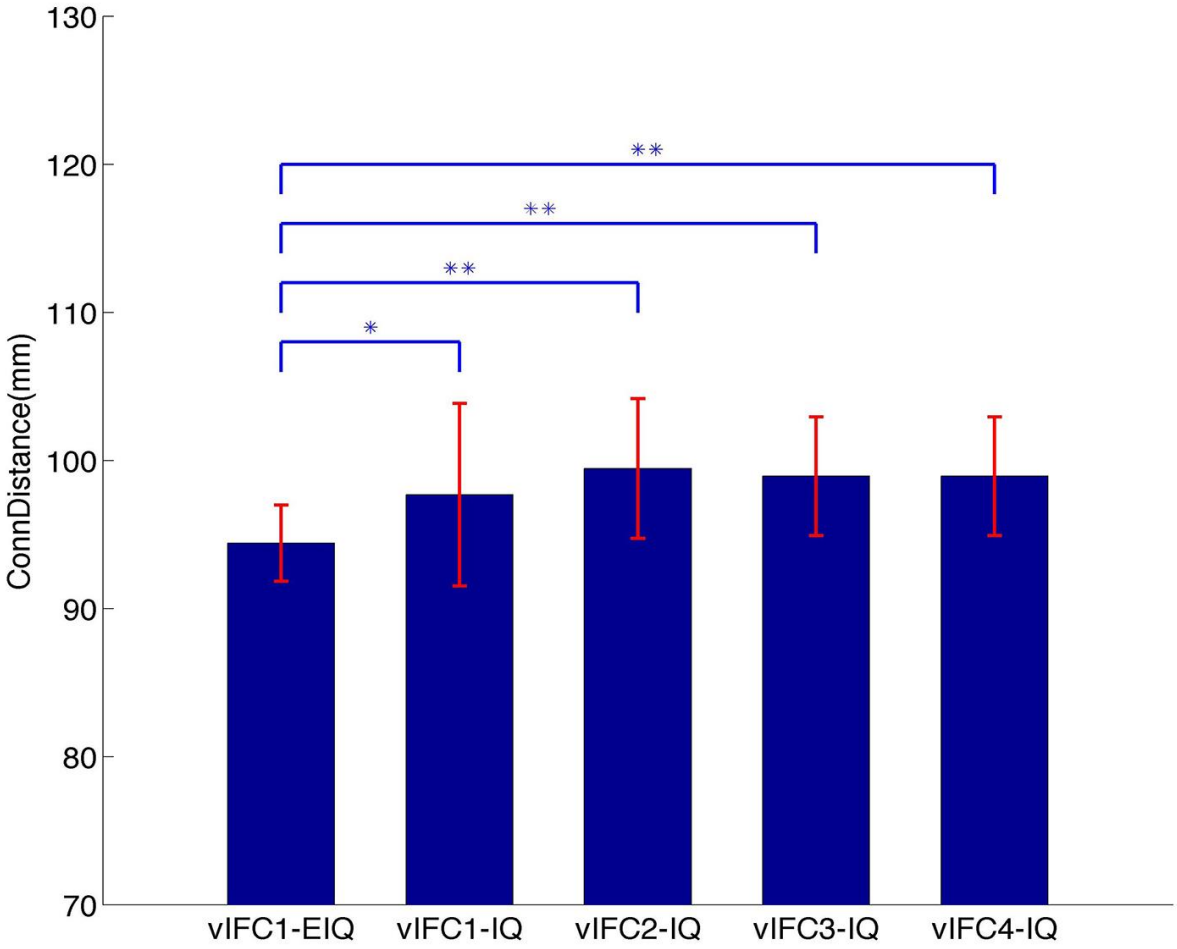
NSFC 11204369
IPCAS Startup Foundation Y1CX222005
973 Program 2015CB351702

Title: Distance-based functional criticality of human brain: Intelligence and emotional intelligence correlates

Authors: *L. JIANG, K. QIAO, C. LI;
Inst. of Psychology Chinese Acad. of Sci., BEIJING, China

Abstract: Vertex-wise Index of Functional criticality (vIFC) of human brain has been proved to be an efficient neuroimaging marker during AD progression and human brain development (Jiang et al., 2018; Jiang et al., 2019). Recently anatomical distance between cortical regions has been testified to play an important role in macaque brain network organization (Ercsey-Ravasz et al., 2013). How does physical distance contribute to functional criticality of human brain as well as behaviors like intelligence and emotional intelligence? Here we used 18 healthy young adults' structural T1 and resting state EPI images (43.1-80.0 years, 10 males) and distance-based functional criticality algorithm to explore the associations between vIFC and behaviors at different spatial scales of brain connectivity. We also defined connectivity distance as the average distance between the significant vIFC-behavior correlation clusters and those vertices with significant functional connectivity. Results show that intelligence and emotional intelligence were related to functional criticality of separate brain regions and didn't have any overlap; intelligence was associated with longer distance and more widespread functional hierarchies compared with emotional intelligence. Our findings not only made a linkage between intelligence/emotional intelligence and functional criticality, but also gave individual behaviors a quantitative characterization in terms of inter-areal distance.





Disclosures: **L. Jiang:** None. **K. Qiao:** None. **C. Li:** None.

Poster

252. Connectomics Analytics III

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 252.03/BB33

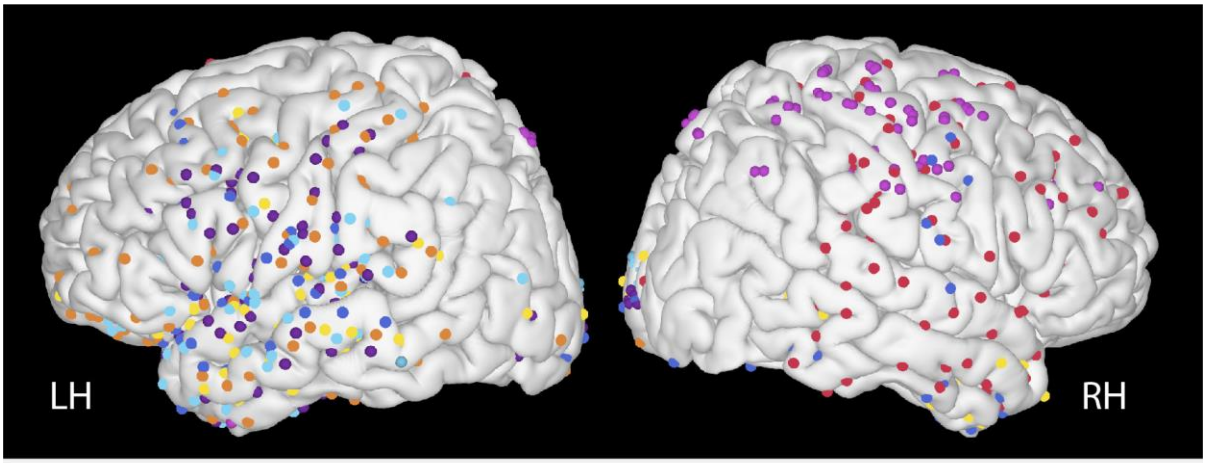
Topic: I.07. Data Analysis and Statistics

Support: NIH R24 MH117529

Title: Group analysis and visualization of intracranial EEG data in RAVE

Authors: *J. F. MAGNOTTI[1], Z. WANG[2], P. KARAS[1], M. LI[2], M. BEAUCHAMP[1]; [1]Neurosurg., Baylor Col. of Med., Houston, TX; [2]Statistics, Rice Univ., Houston, TX

Abstract: Intracranial electroencephalography (iEEG) is a popular technique that allows for direct recording of the activity of small population of neurons from electrodes implanted in the human brain. We have developed RAVE ("R" Analysis and Visualization of iEEG) an open-source, freely-available software package purpose-built for iEEG data. A critical issue in iEEG data analysis is the question of how to generalize results obtained within single electrodes across multiple subjects. A common approach in the field is to use analysis of variance (ANOVA) across electrodes. However, ANOVAs ignore subject-level effects and stimulus exemplar effects by treating each electrode as an independent observation and averaging over all stimulus exemplars within a condition. This approach is referred to as the "fixed-effect fallacy" because it treats all electrodes as if they came from a fictive single subject. As is now well understood in the BOLD fMRI literature, different subjects vary dramatically in their brain responses to identical experimental conditions, so that fixed-effects results can be driven by a single, non-representative subject. A straightforward solution to the fixed-effect fallacy is to use hierarchical models to properly account for the different sources of variation in the data. Here, we describe a module within RAVE that employs linear mixed effects models for handling nested sources of variation in iEEG data. The models account for the fact that individual subjects vary in their response level; within subjects, electrodes in the same brain region will vary in their response level; and different stimuli within a given experimental condition will vary in their ability to evoke a response. Intersubject alignment is accomplished using cortical surface alignment tools implemented in FreeSurfer. Results are visualized on the cortical surface (see Figure). See the RAVE website for more details or to install RAVE: https://openwetware.org/wiki/Beauchamp:RAVE.



Mapping electrodes from 8 subjects to a single cortical surface. Each color represents a single subject.

Disclosures: **J.F. Magnotti:** None. **Z. Wang:** None. **P. Karas:** None. **M. Li:** None. **M. Beauchamp:** None.

Poster

252. Connectomics Analytics III

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 252.04/BB34

Topic: I.07. Data Analysis and Statistics

Support: U01NS103792
R01MH110831
U01NS098961

Title: Cardiac-related modulation of the extracellular action potential *in vivo* reveals multiple cell classes in the human hippocampus

Authors: *C. P. MOSHER[1], Y. WEI[2], J. KAMINSKI[1], A. NANDI[2], A. N. MAMELAK[1], C. A. ANASTASSIOU[2], U. RUTISHAUSER[1];
[1]Dept. of Neurosurg., Cedars-Sinai Med. Ctr., Los Angeles, CA; [2]Allen Inst. for Brain Sci., Seattle, WA

Abstract: Brain circuits that support behavior are composed of many cell classes that can play distinct functional roles. Monitoring these circuits is often achieved *in vivo* via extracellular electrophysiological recordings. For example, genetic manipulation in rodents and quantification of features of the extracellular action potential (EAP) waveform differentiate inhibitory basket cells with narrow fast-spiking (FS) EAP waveforms from excitatory pyramidal neurons with wider, regular-spiking (RS) EAP waveforms. Beyond these cell classes, our understanding of how different cell types support circuit dynamics remains limited. This is particularly true in the human brain as detailed laminar depth recordings are rare and genetic manipulation *in vivo* remains unfeasible.

Here we combine computational modeling of the extracellular field using single-neuron representations of various cell types in human cortex together with *in vivo* microwire recordings in humans to identify features of the EAP waveform that can be used for cell classification. The single-neuron models are generated from *ex vivo*, whole-cell patch-clamp experiments of neurons with full dendritic reconstructions. A computational workflow based on parallelized genetic optimization is used to generate biophysically accurate, single-neuron models with a mixture of Na-, K- and Ca-conductances distributed along the entirety of axonal, dendritic and somatic segments. These models faithfully capture not only intracellular dynamics during spikes but also offer the ability to simulate various aspects of the EAP waveform as a function of single-neuron and cell class characteristics.

We show that the human hippocampus is composed of FS and RS cells and that these cells share similar EAP features with those elicited by aspiny and spiny model neurons. In addition to the classical separation of FS and RS neurons, modulation of the EAP during the cardiac cycle

unveils a new subgroup of RS cells, separating RS classes into RS1 and RS2 subdivisions. While both RS1 and RS2 cells share the same standard features (spike width, amplitude), the EAP waveform varies uniquely with the micrometer-level shifts in recording caused by the heartbeat. RS2 cells share similar EAP features with model spiny neurons that have distinct electrophysiological profiles (lower rheobase current, higher input resistance) from RS1 cells, suggesting these cells belong to a distinct subtype of spiny neuron. Intriguingly, RS2 cells are more likely to synchronize to local hippocampal theta oscillations than RS1 cells, suggesting that they constitute a functional and electrophysiologically unique subclass of neuron in the human hippocampus.

Disclosures: C.P. Mosher: None. **Y. Wei:** None. **J. Kaminski:** None. **A. Nandi:** None. **A.N. Mamelak:** None. **C.A. Anastassiou:** None. **U. Rutishauser:** None.

Poster

252. Connectomics Analytics III

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 252.05/BB35

Topic: I.07. Data Analysis and Statistics

Title: Dynamic causal network analysis of intracranial EEG during a memory task

Authors: *J. KOPAL[1,2,3], J. HLINKA[2,4], E. DESPOUY[1], J. CUROT[1,5], M. DENUELLE[5], L. VALTON[1,5], E. J. BARBEAU[1], J.-C. SOL[5];
[1]Ctr. de Recherche Cerveau et Cognition, Univ. Toulouse III - Paul Sabatier, Toulouse, France; [2]Dept. of Complex Systems, Inst. of Computer Sci. of the Czech Acad. of Sci., Prague, Czech Republic; [3]Dept. of Computing and Control Engin., Univ. of Chem. and Technol., Prague, Czech Republic; [4]Natl. Inst. of Mental Hlth., Klecany, Czech Republic; [5]Univ. Hosp. Purpan, Toulouse, France

Abstract: Our sense of ourselves is grounded in memory and yet how memory works remains a puzzle. This puzzle might be resolved by studying the brain networks underlying memory. Those are represented by spatially distributed, but functionally linked, regions that share information with each other. Understanding memory requires the investigation of the activation of these networks, in particular of the interactions between different areas while memory processes unfold. Such interactions can be described in form of Granger causality, which is helpful to analyse the synchronization and information flow between neural circuits. We recorded the brain activity of 18 epileptic subjects (8 females, mean age 37,61 ± 11,37) using multiple intracranial depth electrodes targeting different brain areas, including subcortical regions. During data recording, each of the subjects underwent a speeded visual recognition memory task. The main disadvantage of such recordings is the difficulty of summarizing results across individual subjects. We propose to use non-negative matrix factorization as a tool for data integration,

thanks to which distinct underlying memory networks can be described at various time points. During early stages of the test (0-350 ms) we found increased amount of connections in the left hemisphere. Conversely, during late stages of the test (350-600 ms) more connections in the right hemisphere were present. These connections were mixture of feedforward and feedback links within a given hemisphere, which included structures of the medial temporal lobes, namely the hippocampus, parahippocampal, fusiform and temporal gyrii. These played the main role in the memory networks. We also found that the memory networks supports specific abilities because they showed decreased efficiency compared to corresponding random networks. Quantifying diverse aspects of network organization during the memory process we can bring new insight on how different brain areas coordinate their activities.

Disclosures: **J. Kopal:** None. **J. Hlinka:** None. **E. Despouy:** None. **J. Curot:** None. **M. Denuelle:** None. **L. Valton:** None. **E.J. Barbeau:** None. **J. Sol:** None.

Poster

252. Connectomics Analytics III

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 252.06/BB36

Topic: I.07. Data Analysis and Statistics

Support: KAKENHI JP 26870934
SASAKAWA SCIENTIFIC RESEARCH GRANT

Title: Revealing hierarchical relationships among cognitive functions as a concept lattice using formal concept analysis

Authors: *H. KURASHIGE[1,2], Y. YAMASHITA[2], R. OSU[4], Y. OTAKA[5,6], T. HANAKAWA[3], M. HONDA[2], H. KAWABATA[7];
[1]Inst. of Innovative Sci. and Technol., Tokai Univ., Tokyo, Japan; [2]Natl. Inst. of Neurosci., Natl. Ctr. of Neurol. and Psychiatry, Tokyo, Japan; [3]Integrative Brain Imaging Ctr., Natl. Ctr. of Neurol. and Psychiatry, Kodaira, Japan; [4]Fac. of Human Sci., Waseda Univ., Tokorozawa, Japan; [5]Dept. of Rehabil. Med. I, Shool of Medicine, Fujita Hlth. Univ., Aichi, Japan; [6]Dept. of Rehabil. Med., Tokyo Bay Rehabil. Hosp., Chiba, Japan; [7]Dept. of Psychology,, Keio Univ., Tokyo, Japan

Abstract: Thanks to the continuous efforts in the field of functional brain imaging, we have identified brain regions supporting each cognitive function. Since we can consider that groups of cognitive functions for which similar brain regions are responsible are interrelated each other in the underlying mechanisms, accumulation of brain imaging data enable us to construct relational knowledge comprehending many cognitive functions as a unity. Indeed, several meta-analytic studies have shown latent mechanisms commonly supporting superficially separated cognitive

functions. In the present study, we tried to reveal hierarchical relationships among dozens of the cognitive functions (e.g. “working memory”, “emotion”, “episodic memory”, etc.) as a concept lattice using formal concept analysis. A lattice is a partially ordered set (poset) that possess least upper bound and greatest lower bound for any pair of elements in the set. A power set of a finite set with order naturally defined by inclusion relation is one of the typical lattices. A concept lattice has tuples of subsets of *objects* and subsets of *attributes* as the elements. The order of the concept lattice is introduced as inclusion between subsets of objects. Equivalently, the order is also introducible as inclusion between subsets of attributes. Therefore, these orders are dual. In this study, we considered cognitive functions as objects. Additionally, we considered brain regions or components (calculated based on data from large-scale fMRI database) as the attributes. We made an association between the objects (cognitive functions) and attributes (brain regions or components) based on the large-scale fMRI database. Then we obtained the concept lattices with tuples of the objects and attributes as the elements using the formal concept analysis. Most importantly, the resultant concept lattices show a hierarchy of cognitive functions where they are ordered based on the inclusions between the subsets of the attributes possessed by them. For instance, we found that “meaning” is included in “comprehension” and “comprehension” is included in “concept”. We consider that this analysis brings a comprehensive view of brain functions and useful insights into the dysfunction of cognitive abilities.

Disclosures: H. Kurashige: None. **Y. Yamashita:** None. **R. Osu:** None. **Y. Otaka:** None. **T. Hanakawa:** None. **M. Honda:** None. **H. Kawabata:** None.

Poster

252. Connectomics Analytics III

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 252.07/BB37

Topic: I.07. Data Analysis and Statistics

Title: Neuromuscular adaptations associated with muscle fatigue during the 6-minute walk test

Authors: *M. BIELMANN, M. BERTRAND-CHARETTE, G. GOURDE, J.-S. ROY, *L. J. BOUYER;
CIRRIS, Univ. Laval, Quebec, QC, Canada

Abstract: BACKGROUND. Early detection of muscle fatigue during movement execution would be a useful tool for injury prevention as well as during motor rehabilitation. During static muscle contractions, the shift in the median frequency of the EMG power spectrum (MDF-shift) has been proposed as a good indicator of neuromuscular adaptation associated with muscle fatigue. However, during complex movement, reliable detection of muscle fatigue becomes very challenging due to changes in EMG pattern and amplitude of activity and few data processing

methods have been validated to date. The objective of the current study was to validate if MDF-shift can be used to as an early indicators of muscle fatigue during the 6-minute walk test (6MWT), a standardized functional test used to assess endurance during gait.
METHODS. 30 healthy participants performed a 6MWT before and after completing a muscle fatigue protocol consisting of repetitive ankle dorsiflexions to the pace of a metronome. Neuromuscular adaptations were quantified by a decrease in the median frequency of the power spectrum of electromyographic activity (EMG), recorded during gait using wireless EMG amplifiers (Delsys Trigno). Degradation in the kinematic walking pattern was also measured, using an algorithm of cross correlation between a template of joint angular excursion (created from 30 to 60 baseline strides) and individual gait cycles.
RESULTS. A significant drop in median frequency (25 ± 8%; $p<0.05$) post fatigue exercise was observed specifically in the tibialis Anterior muscle (TA), indicating the presence of muscle fatigue. A significant degradation of the kinematic walking pattern was also observed at the ankle post fatigue ($p<0.05$). Furthermore, in post-fatigue, subjects recovered on average after 7.88 ± 8.58s for kinematic data and 156.7 ± 129.8s for EMG.
DISCUSSION. The use of wearable sensors and the adaptation of laboratory analysis methods, such as shifts in EMG median frequency and decreases in kinematic walking pattern coherence can be an efficient approach to detect small reductions in movement quality and the onset of neuromuscular adaptation “outside of the laboratory”. The results of this study demonstrate that it is not only possible to recover during walking after muscle fatigue exercise, but that the kinematic values recover faster than those of EMG. The decrease in MDF suggests a reorganization in the activation of type II (fatigable) motor units to Type I motor units (fatigue-resistant). As this occurs after kinematic recovery, this neuromuscular compensation is functionally effective.
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Poster

252. Connectomics Analytics III

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 252.08/BB38

Topic: I.07. Data Analysis and Statistics

Support: Whitehall Foundation 2017-12-73
NSF Grant 1736028

Title: Comparing the effects of pre-stimulus periodic and aperiodic activity on post-stimulus event-related potentials

Authors: *F. ZHANG, T. DONOGHUE, B. VOYTEK;
Cognitive Sci., UC San Diego, La Jolla, CA

Abstract: A common experimental approach in cognitive and behavioral neuroscience is to present stimuli in order to measure and interpret subsequent evoked responses; for example, examining event-related potentials (ERPs) in electroencephalography (EEG). These evoked potentials are often analyzed based on their amplitude and latency in relation to experimental conditions of interest, and normalized to pre-stimulus activity. However, prior work has highlighted how this pre- or peri-stimulus brain activity can explain variance in subsequent ERP sizes—that is, the brain state at the moment of stimulus presentation can sometimes predict subsequent behavioral and neural responses. Such analyses have typically focused on periodic, or oscillatory, activity, such as the endogenous alpha rhythm. However, prior work has shown that neuro-electrophysiological data includes a 1/f-like component—which we refer to as aperiodic activity—wherein the power decreases with increasing frequency. Changes in this aperiodic activity have been shown to be correlated with age and working memory performance, indicating it has behavioral significance. In this study, we further explored the effects of pre-stimulus activity by examining the aperiodic activity, and comparing it to periodic activity, to see how each predicts subsequent ERPs. We use non-invasive scalp EEG recordings of participants engaged in visual psychophysical tasks, and analyze resulting ERP amplitudes and latencies, including for the P1, N2, and P3 components. We use a recently developed algorithm to parametrize the periodic and aperiodic activity in neural power spectra (Haller et al, 2018), calculated on a per-trial basis on pre-stimulus activity, and compare these measures to subsequent evoked responses. We replicate known effects of pre-stimulus alpha power and phase on subsequent ERP amplitude. In addition, when examining aperiodic properties, we find that at the group level, there is not a consistent effect of pre-stimulus aperiodic activity on subsequent ERPs. This suggests that while ongoing oscillations do have significant effects on subsequent responses, trial-by-trial variations in the aperiodic activity does not have a consistent effect. We follow up on the group level analysis by exploring individual differences. The results from these analyses raise questions about the relationship between different components of ongoing activity, and subsequent evoked responses.

Disclosures: F. Zhang: None. **T. Donoghue:** None. **B. Voytek:** None.

Poster

252. Connectomics Analytics III

Location: Hall A

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Program #/Poster #: 252.09/BB39

Topic: I.07. Data Analysis and Statistics

Support: JSPS KAKENHI Grant Number 17K01992

Support Center for Advanced Telecommunications Technology Research Foundation

Title: Classification of working memory contents using estimated cortical currents from EEG data during N-back task

Authors: *K.-I. MORISHIGE[1], Y. GAMANO[2], H. TAKANO[1], K. IDO[3];
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Abstract: Electroencephalography (EEG) studies of working memory have demonstrated cortical activities and oscillatory representations but have not clarified what kind of information is stored in memory representations. To answer this question, we measured scalp EEG and fMRI data while participants (two males, 21 and 23 years old) performed a N-back working memory task. This task consists of three periods: (1) an encoding period, where six arrow stimuli, chosen from four types of arrows (left, right, up, and down) with replacement, were presented sequentially on a monitor. One of the stimuli was randomly presented with a red-color arrow. The participants were instructed to memorize the arrow direction that appeared two steps before the red-color arrow; (2) a retention period, during which this information was maintained over a brief delay; and (3) a response period, in which the participants judged whether a probe arrow direction matched the retained one. Various kinds of artifacts, such as eye movements, contaminated the measured EEG data. In order to remove the artifacts, we employed an extra-dipole method that is based on a hierarchical Bayesian method and simultaneously estimates the cortical and eye currents by solving the EEG inverse problem using fMRI data as prior information. We calculated the current intensities from the estimated cortical currents. The cortical regions of the prefrontal cortex, the intraparietal sulcus, and the middle temporal gyrus had large current intensities. These areas are known to be related to working memory processes. To investigate the representation of working memory on the cortical regions, we attempted to classify information about the contents of the working memory using the estimated cortical currents. We divided the currents in the retention period into ten subperiods and calculated the time average values for all subperiods and dipoles. We used a sparse logistic regression to reduce the input dimensions of the time average values and classified them into true or false answer groups. As a result, classification accuracy was 77.19±2.26%. Additionally, we also classified them into the four arrow direction groups. Classification accuracy was 63.82±6.40%. In both classification results, the weight values were distributed on multiple cortical areas related to working memory functions. These results indicate that our method is able to classify, to some extent, information about the contents of working memory and that the amplitude of EEG cortical currents over multiple regions contributes to working memory functions.

Disclosures: **K. Morishige:** None. **Y. Gamano:** None. **H. Takano:** None. **K. Ido:** None.

Poster

252. Connectomics Analytics III

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 252.10/BB40

Topic: I.07. Data Analysis and Statistics

Support: We wish to thank the Allen Institute for Brain Science founder Paul G. Allen for his vision, encouragement and support.

Title: Conserved and divergent electrophysiological and morphological properties of mouse and human transcriptomically-defined cell types

Authors: *A. BUCHIN[1], T. CHARTRAND[1], T. BAKKEN[1], J. MILLER[1], R. HODGE[1], N. GOUWENS[1], S. GRATIY[1], B. KALMBACH[1,2], B. TASIC[1], C. LEE[1], J. LEE[1], G. MURPHY[1], H. ZENG[1], J. TING[1], S. SORENSEN[1], J. BERG[1], E. LEIN[1,2], C. A. ANASTASSIOU[1,3]; [1]Allen Inst. For Brain Sci., Seattle, WA; [2]Univ. of Washington, Seattle, WA; [3]Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Gene expression, electrophysiological and morphological studies suggest that the neocortex consist of various cells types. There is increasing evidence that a number of homologous cell types are preserved over the evolution of the mammalian cortex. To characterize the convergent and divergent properties of single neurons, we performed a comparative analysis of the electrophysiological and morphological features of mouse and human neurons according to homologous cell types as defined by transcriptomics (Hodge & Bakken et al. 2018). We utilized data from the large-scale patch-seq pipelines focused on the mouse visual cortex and human middle temporal gyrus, with tissue derived from epilepsy and tumor patients. We compared inhibitory fast-spiking interneurons and found that 50 electrophysiological features out of 96 and 6 morphological features out of 40 differed significantly between mouse and human. These results suggest that certain electrophysiological features were preserved during the evolution, while others became substantially divergent. Overall our results show the large-scale and multi-modal comparative cross-species analysis between homologous cortical cell types, indicating the specific morphological and electrophysiological properties of the human neurons. We speculate about how such across-species, within-cell type differences in neural characteristics alter their function and support computations.

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Poster

252. Connectomics Analytics III

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 252.11/BB41

Topic: I.07. Data Analysis and Statistics

Title: Depth- and size-dependent intrinsic membrane properties of human supragranular pyramidal cells: Functional predictions in biophysical models from cross-modality single cell data

Authors: *T. CHARTRAND[1], A. NANDI[1], A. BUCHIN[1], C. KOCH[1], E. S. LEIN[1,2], B. E. KALMBACH[1,3], C. A. ANASTASSIOU[1,4];
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Abstract: Recent experimental observations have shown that supragranular pyramidal cells in human cortex exhibit significant diversity in their intrinsic properties, often correlated with depth from the cortical surface. Whether such gradients serve distinct computational roles for pyramidal cells by depth, compensatory changes to maintain function across different morphologies, or a combination of these explanations remains an open question.
At the Allen Institute a high-throughput platform has been established generating electrophysiological, morphological and genetic single-neuron data from human cortex (medial temporal gyrus) of epilepsy and tumor patients undergoing brain surgery. From such data it is observed that cell size and related morphological properties vary significantly with depth, necessitated by the significant expansion of the superficial layers of human cortex. Moreover, similar trends are found in the expression of ion channel-associated genes including the h-channel (HCN), known to affect resonance properties and propagation of dendritic input [Kalmbach et al., Neuron, 2018].
To explore why different electrophysiological and morphological single-neuron features co-vary with cortical depth, we generated biophysically detailed models optimized to match paired electrophysiology and morphology data of individual human pyramidal cells. The models show an increase in h-channel conductance with depth that matches gene expression data. We test the model responses to a range of in vivo-like synaptic inputs and assess which response properties correlate with cell size and depth. To distinguish compensatory from functional differentiation, we compare experimental correlations for somatic input properties to predicted correlations for dendritic input, with a reduced correlation for dendritic responses indicating compensation for changes in dendritic morphology. We investigate the mechanisms for this compensation by in-depth analysis of model responses, and also discuss how related size-dependent effects are reflected in a cross-species comparison of mouse and human pyramidal cells.

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Poster

252. Connectomics Analytics III

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Program #/Poster #: 252.12/BB42

Topic: I.07. Data Analysis and Statistics

Support: NSF 1736028
Whitehall Foundation

Title: Dissociating contributions of periodic and aperiodic neural activity in human visual working memory

Authors: *Q. VAN ENGEN[1,2], G. CHAU[6], B. VOYTEK[7,3,4,5];
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Abstract: Ample evidence exists demonstrating the contributions of periodic, oscillatory neural activity to visual perception as well as working memory maintenance and recall. In particular, occipital alpha (8-12 Hz) and midline frontal theta (4-8 Hz) oscillations have been shown to parametrically track the number of items held in working memory, as well as later successful recall. However, emerging evidence is showing the importance that aperiodic, non-oscillatory, neural activity plays in cognition and behavior. This aperiodic activity—which has been linked to contributions of postsynaptic excitation and inhibition—has a 1/f-like distribution in the neural power spectral density. Additionally, alterations in the aperiodic signal can be mistaken for event-related changes in oscillatory activity, where no such changes to the oscillation necessarily occur. Here, we seek to examine to what degree well-described alpha and theta correlates of visual working memory are actually derived from oscillatory, as opposed to non-oscillatory aperiodic, activity changes. To do this we reanalyzed EEG data from a Visual Working Memory task (Adam *et al*., 2018) using an approach that quantifies both the aperiodic components of a power spectrum, as well as true oscillatory power within a frequency band. First, we find that all 31 subjects in Experiment 1, and 37/44 of subjects in Experiment 2, show some degree of occipital alpha activity, while only 18/44 show some degree of midline frontal theta. This suggests that group-level analyses of midline frontal theta is frequently measuring just the aperiodic signal, and not actual oscillatory changes. In contrast, and in line with past work, there is a clear suppression of visual cortical alpha power, with more suppression with increasing memory load. In addition, there was also an effect of memory load both the aperiodic exponent

(slope) and offset. Higher set-sizes correspond to “flatter” spectral slopes than lower set-sizes, drive by a general decrease in power of lower frequencies and an increase in higher frequencies. More surprisingly, past work shows that midline frontal theta power is stronger for better performers. Here, using the same data, we show a decrease in absolute theta power for both good and poor performance during the retention period, compared to baseline. Furthermore, theta power *relative to the aperiodic signal* is higher during the retention period for good performance, compared to poor performance. Thus, narrowband filtering always filters both true theta power and the aperiodic signal, but cannot disentangle them which could lead to misinterpretations of the true neural activity change.

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Poster

252. Connectomics Analytics III

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Program #/Poster #: 252.13/BB43

Topic: I.07. Data Analysis and Statistics

Title: A content analysis of data analytic reporting practices in cognitive neuroscience journals

Authors: *A. A. CONLEY, T. K. LAFFERTY, T. C. HATVANY, J. D. GRIFFITH; Shippensburg Univ., Shippensburg, PA

Abstract: The purpose of this study was to conduct a content analysis on the reporting practices by authors in Cognitive Neuroscience journal articles. Prior research has conducted content analyses on reporting practices and statistical analyses used in different fields in an effort gain a better understanding of current trends. The sample for this study consisted of research articles from journals in cognitive neuroscience. Journals were initially identified in the Scientific Journal Rankings database as the field of cognitive neuroscience. Specifically, journals were examined in terms of the reported 1-year impact factor scores for 2017 reported in the database. The database provided quartiles which was used in the current study. In an effort to examine a wide range of journals, two journals per quartile were randomly selected and every article from the selected journals for the year 2018 was retrieved. This yielded a sample of 438 articles. Articles were initially coded on the basis of if it was empirical/quantitative in nature. Thus, those not coded as empirical/quantitative may have been reviews, commentaries, book/test reviews, theory/review papers, simulation studies, or qualitative studies. The primary interest was to focus on the reporting practices and statistical analyses used within the field for empirical/quantitative studies. A total of 286 (65.3%) were coded as empirical/quantitative in nature. Each of the articles that met the criteria for an empirical/quantitative study was then coded across a series of variables that included: if a power analysis was conducted, number of experiments, sample size, statistical analyses used, number of different statistical analyses, and the number of tables and

figures. The content analysis revealed that 3.2% of studies did perform a power analysis. The number of experiments ranged from 1 to 5 with a mean of 1.2 (SD = .56) and the mean sample size was 159.4 (SD = 729.32). The five most common statistical analyses in descending order were correlations, t/z-tests, ANOVA, chi-square, and multiple regression. The mean total number of analyses conducted per study was 31.9 (SD = 58.34). Lastly, there was a mean of 2.3 (SD = 2.32) tables and 4.4 (SD = 2.70) figures per study. Two concerns of the study were a lack of power analyses and the large number of different analyses conducted per study and Type I error considerations. Researchers in this field come from a variety of different training backgrounds and it is important to have an understanding of the current reporting practices in current research.

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Poster

252. Connectomics Analytics III

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Program #/Poster #: 252.14/BB44

Topic: I.07. Data Analysis and Statistics

Support: U01MH114812
1RF1MH114126-01
VA-BLR&D Merit Review 821-MRNB-24218
NIH NS044163

Title: Intrinsic membrane properties, morphology and transcriptomic profile of a rare human L5-projection neuron type

Authors: *B. E. KALMBACH[1,2], R. DE FRATES[1], L. GRAYBUCK[1], T. DAIGLE[1], P. CHONG[1], X. OPITZ-ARAYA[1], M. WALKER[1], J. BERG[1], N. C. DEMBROW[2], W. SPAIN[2], G. HORWITZ[2], C. COBBS[3], R. ELLENBOGEN[4], R. GWINN[3], A. KO[4], J. G. OJEMANN[4], D. SILBERGELD[4], B. TASIC[1], E. LEIN[1,4], J. TING[1,2];
[1]Allen Inst. For Brain Sci., Seattle, WA; [2]Univ. of Washington Dept. of Physiol. and Biophysics, Seattle, WA; [3]Swedish Neuoscience Inst., Seattle, WA; [4]Dept Neurosurg., Univ. of Washington, Seattle, WA

Abstract: Layer 5 (L5) projection neurons in rodent neocortex can be broadly segregated into two groups: Neurons with axonal projections restricted to the telencephalon (Intratelencephalic-projecting or IT) and neurons with axonal projections both inside and outside of the telencephalon (Extratelencephalic-projecting or ET). Assaying the cellular properties of these neuron populations in humans is challenging because 1) these neurons are defined by their long-range projections and 2) current evidence suggests that L5 ET neurons are rare across various

human neocortical brain regions. Here we use a multipronged approach to study ET and IT neuron populations in human *ex vivo* neocortical tissue and to test whether they possess qualitatively similar cellular properties as observed for these L5 neuron types in rodent. First, we performed patch clamp recordings from human L5 pyramidal neurons in *ex vivo* neocortical brain slices derived from neurosurgeries. Clustering neurons based on intrinsic membrane properties revealed two broad neuron classes: those with physiological properties resembling rodent ET neurons and those resembling rodent IT neurons. These physiologically defined cell types also exhibited distinct morphological features. Direct dendritic recordings from putative ET neurons revealed strong Ca^{2+}electrogenesis, a hallmark of this neuron type in many sensory areas of rodent neocortex. To corroborate these findings we performed patch-seq experiments (combined physiological and transcriptomic analysis from the same cell) to assign a transcriptomic cell type identity to these physiologically defined cell types. Physiologically defined human L5 ET neurons mapped to a human transcriptomic cluster corresponding to mouse L5 ET neurons whereas putative human L5 IT neurons mapped to transcriptomic clusters corresponding to L5 IT types. Thus, as in rodent neocortex, L5 pyramidal neurons in the human neocortex are segregated into two broad projection types with distinctive transcriptomic, morphological and physiological properties. Finally, we demonstrate that an ET-specific enhancer element proximal to the mouse Fam84b gene (a known marker gene of the L5 ET neuron type) can be leveraged in an AAV vector to achieve strong enrichment of L5 ET neuron labeling in human and non-human primate brain *ex vivo* slice cultures. This strategy labels putative ET neurons across multiple primate neocortical regions, including putative cortico-spinal neurons and Betz cells in motor areas. These data establish a roadmap for studying diverse, genetically-defined, deep-layer projection neurons across many cortical areas in the human and non-human primate.

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Poster

252. Connectomics Analytics III

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Program #/Poster #: 252.15/BB45

Topic: I.07. Data Analysis and Statistics

Title: The efficacy of the deep learning approach for generalization of the seizure prediction algorithm

Authors: *S. YAMAMOTO[1], T. YANAGISAWA[3], R. FUKUMA[2], H. KISHIMA[2];
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Abstract: *Background* Quality-of-life [QOL] of patients with epilepsy are mostly impaired because of the uncertainty of seizure occurrence. This is especially true in 30% of these patients, in whom the seizures are insufficiently controlled even with extensive medical or surgical treatment. Accurate seizure prediction may help to improve the QOL of these patients by providing a chance to avoid unexpected danger resulted from their seizures. Compared to non-invasive signals such as EEG and MEG, the signal to noise ratio is higher in invasive electrocorticography. Seizure prediction using electrocorticography [ECoG] in previous studies appeared promising. However, the algorithm, or decoder, was trained for each patient. It was difficult to apply a decoder for another patient. We hypothesized that some common important features in ECoG signals of epileptic seizures could predict seizure of any patients. We aim to demonstrate that a deep learning [DL], which automatically extracts a set of new features, predicts seizures of patients, whose data is not used in the algorithm development, with better accuracy compared to the Linear Support Vector Machine [SVM] with some commonly-used-features, spectral power.
Method One hundred and five seizures recorded in 20 patients with epilepsy who underwent ECoG study for presurgical evaluation at Osaka University Hospital were included. Data were recorded at 10kHz and down sampled to 2kHz. Data from both preictal and interictal periods of all the patients selected as follows were then subjected to the machine learning algorithms: 2940 1-sec segments from the preictal periods 200-1700ms before seizure onset with overlaps and the same amount of randomly selected 1-sec segments from the interictal periods. For DL model, we used Recurrent Neural Network [RNN] to classify these two datasets. We evaluated the performance of RNN model using 5-fold cross-validation. As a comparison model, we used SVM to classify the same datasets by utilizing the spectral power of eight frequency bands (δ: 1-3 Hz, θ: 3-8 Hz, α: 8-13 Hz, β: 13-25 Hz, low γ: 25-50 Hz, and high γ: 70-110 Hz, high frequency oscillation: 110-250 Hz, 250-500Hz) in each segment. We then compared the accuracy of the two models.
Results The mean classification accuracy was 64.2% for RNN and 56.5% for SVM. The performance of the RNN was significantly better than SVM ($p < 0.001$).
Discussion RNN outperformed SVM in classifying preictal and interictal signals in the electrocorticography of patients with epilepsy. This suggested that RNN may have extracted some new features other than spectral power, which were useful in improving the generalizability of the classification model in new dataset.

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Poster

252. Connectomics Analytics III

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Topic: I.07. Data Analysis and Statistics

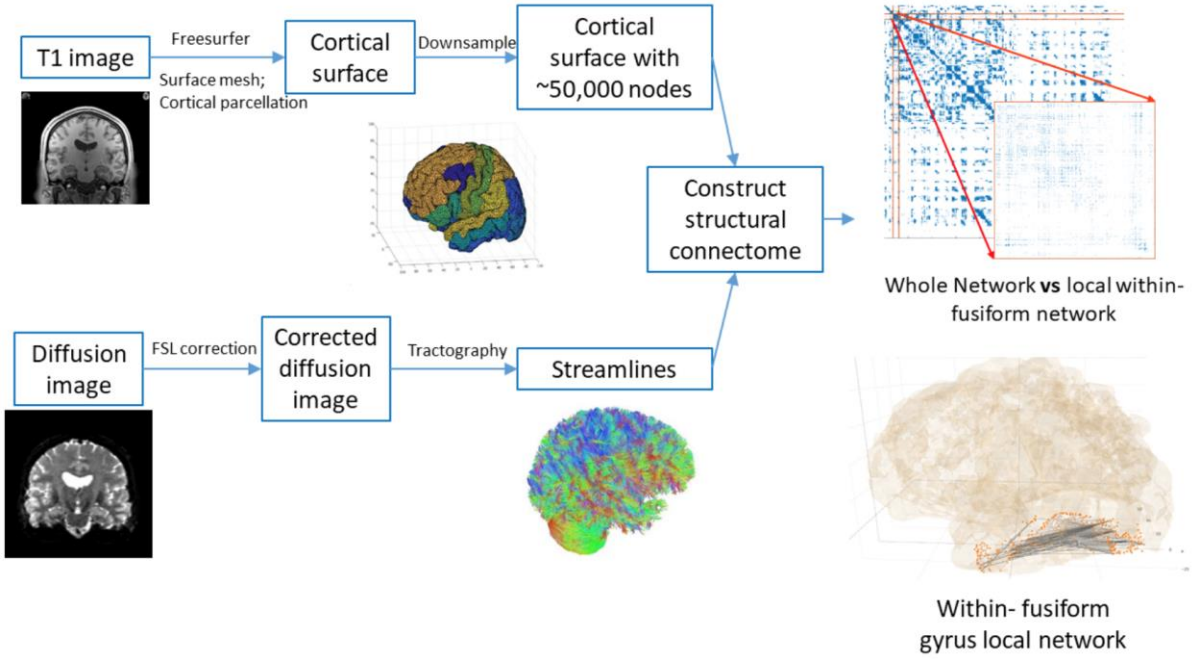
Support: China Scholarship Council No. CSC201706450045
EPSRC (NS/A000026/1)
Guangci Professorship Program of Ruijin Hospital (Shanghai Jiao Tong University)
Wellcome Trust (102037)

Title: Altered within-region local organization in temporal lobe epilepsy based on high-resolution structural connectivity

Authors: X. CHEN[1,2], Y. WANG[2], S. KOPETZKY[3,4], **M. BUTZ-OSTENDORF**[3], P. TAYLOR[1], *M. KAISER[1,5];
[1]Newcastle Univ., Newcastle upon Tyne, United Kingdom; [2]Sch. of Information and Control Engin., China Univ. of Petroleum (East China), Qingdao, China; [3]Biomax Informatics AG, Planegg, Germany; [4]Genome-oriented Bioinformatics, TUM Sch. of Life Sci., Munich, Germany; [5]Sch. of Med., Jiao Tong Univ., Shanghai, China

Abstract: Global structural connectivity between brain regions varies between patients suffering from brain disorders. As a consequence, clear differences to healthy controls are often difficult to establish or only are only apparent at later stages of a disease. Here, we observe whether changes at the local level, affecting connections within brain regions, can be a biomarker of brain diseases. We constructed high-resolution structural networks with around 50,000 nodes based on deterministic tracking of diffusion tensor imaging data from healthy subjects and temporal lobe epilepsy patients. Nodes were allocated to 68 cortical regions according to the Desikan-Killany (DK) atlas. As a result, each cortical region contained hundreds of nodes and their intra-areal connections. Several graph metrics—edge density, clustering coefficient, local efficiency, global efficiency, and small-worldness—were calculated for each cortical region network. The NeuroXM Brain Science Suite (http://www.biomax.com/neuroxm) was used for MRI processing, network reconstruction, and visualization of the network differences between controls and patients. We found that edge density and clustering coefficient for patients were lower within regions using the high-resolution network (~50,000-node), whereas no significant difference for the above two metrics were observed in low-resolution networks (68-node) between regions. Moreover, local changes occurred for the fusiform gyrus and lateral orbitofrontal cortex in patients. In addition, the number of regions showing local changes increased with disease duration. As a proof of concept, this shows that local connectivity within regions could be a

novel biomarker for epilepsy diagnosis but potentially also for planning interventions such as deciding which region to surgically remove for medically intractable epilepsy.



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Poster

252. Connectomics Analytics III

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Program #/Poster #: 252.17/BB47

Topic: I.07. Data Analysis and Statistics

Title: *In vivo* cortical circuit characterization in a large-animal model through massively parallel extracellular Neuropixels recordings

Authors: ***Y. WEI**[1], D. J. DENMAN[1], I. KIM[2], V. KREMEN[2], S.-Y. CHANG[2], H. J. JO[2], C. KOCH[1], G. A. WORRELL[2], C. ANASTASSIOU[1];
[1]Allen Inst. for Brain Sci., Seattle, WA; [2]Mayo Clin., Rochester, MN

Abstract: A powerful new extracellular *in vivo* recording probe was recently introduced able to record hundreds of units across several cortical layers in rodents (Jun et al, *Nature*, 2017). The sub-ms temporal resolution of silicon probes has historically been the main means toward understanding the neural code *in vivo* (Buzsaki, *Nat Neurosci*, 2004). Despite progress, the same type of recordings has been difficult to translate to humans. As a result, the cellular and network

mechanisms giving rise to high-level cognition in humans have remained largely speculative. To address these challenges and offer a path for the use of Neuropixels in humans for carefully selected patients and appropriate clinical situations, we have assembled a multi-disciplinary team comprised of electrophysiologists, computational neuroscientists, clinicians, and neurosurgeons. Critical to establishing safe procedures is testing the experimental workflow and Neuropixels deployment in a large-animal better matching human brain characteristics in term of anatomy as well as within surgery-circumstances (e.g. respiratory motion artifact, etc.). In this study, for the first time, we used Neuropixels probes in porcine brains to simultaneously record neuronal activity across different brain regions during sensory stimulation (such as visual or snout stimulation). We monitored the activity of local field potentials (LFPs) as well as the spiking of isolated single units. We compared the stimulation triggered LFP, current source density (CSD), as well as spike activity, before, during, and after stimulation. We monitored from tens to hundreds units per shank in the porcine brain which, in turn, allowed detection of functional connectivity clusters as well as monitoring their evolution as function of brain state and sensory stimulation. Finally, we combined Neuropixels recordings with high-density multi-scale electrocorticographic recordings in an attempt to bridge between cortical depth processing and surface signals. The application of Neuropixels probes in a large animal model clearly exhibits its high promise toward studying cortical dynamics, functional connectivity and, even, short-term dynamics in human cortex.

Disclosures: **Y. Wei:** None. **D.J. Denman:** None. **I. Kim:** None. **V. Kremen:** None. **S. Chang:** None. **H.J. Jo:** None. **C. Koch:** None. **G.A. Worrell:** None. **C. Anastassiou:** None.

Poster

252. Connectomics Analytics III

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 252.18/BB48

Topic: H.02. Human Cognition and Behavior

Support: NSF CAREER 1752355 to JRW
NIH R01 NS102201 to JRW
INI Research Program of Excellence funds to JRW

Title: Disentangling motor inhibition and attentional detection in rIFC lesion patients with Bayesian hierarchical approach

Authors: *Y. CHOO[1], D. MATZKE[2], M. BOWREN[1], D. TRANEL[1,3], J. R. WESSEL[1,3]; [1]Dept. of Psychological & Brain Sci., Univ. of Iowa, Iowa City, IA; [2]Dept. of Psychology, Univ. of Amsterdam, Amsterdam, Netherlands; [3]Dept. of Neurol., Univ. of Iowa Hosp. and Clinics, Iowa City, IA

Abstract: An important unanswered question in cognitive neuroscience is how to countermand already initiated responses when they are no longer necessary. A key process involved in successful action-stopping is motor inhibition, which is frequently studied utilizing the stop-signal task. In the current study (preregistered on 10/26/2018, Open Science Forum, https://osf.io/d9r4s/), we investigated whether increased stopping latencies (stop-signal reaction time, SSRT) previously found in patients with right inferior frontal cortex (rIFC) lesions performing the stop-signal task (Aron et al., 2003) are partially due to attentional deficits manifesting as failures to trigger the stop process. This question stemmed from two main considerations: the inherent characteristics of the SSRT estimate and the role of rIFC during stopping. First, it is unclear whether increased SSRT is indicative of attentional or stopping deficits, because motor inhibition is only the last stage of stopping-related processing, which also includes the initial attentional detection of a stop-signal. Second, because of the often-observed activation of rIFC during saliency detection in other cognitive tasks, the debate on the role of rIFC is still ongoing. In order to resolve these concerns, a Bayesian hierarchical approach (BEESTS, Matzke et al., 2013, 2017) will be applied to stop-signal task data from three different groups: patients with rIFC lesions, brain-damaged comparison patients, and age-, sex-, and handedness-matched healthy comparisons. The BEESTS model allows to calculate attentional and inhibitory deficits separately by quantifying the probability of stop trigger failure. Currently, we report descriptive statistics for the rIFC group (N = 13 out of the targeted sample size of 21) and healthy comparison group (N = 9). While Go RT was virtually identical between both groups (602ms and 614ms for rIFC lesion and healthy comparisons, respectively), SSRT was higher in rIFC lesion group (326ms) than healthy comparisons (239.8ms), which is in line with prior research. No inferential statistics or modeling results are reported at this point as the full sample indicated in the pre-registration is not yet collected (data collection is slated to be complete prior to the conference). Ultimately, by applying BEESTS model to the full set of data, our findings should help resolve the role of rIFC in attentional detection and motor inhibition during action-stopping.

Disclosures: Y. Choo: None. **D. Matzke:** None. **M. Bowren:** None. **D. Tranel:** None. **J.R. Wessel:** None.

Poster

252. Connectomics Analytics III

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 252.19/BB49

Topic: I.07. Data Analysis and Statistics

Title: The Oscillatory ReConstruction Algorithm (ORCA) adaptively detects and quantifies neural oscillations aross species

Authors: *A. WATROUS[1], R. J. BUCHANAN[2];
[1]Univ. of Texas at Austin, Austin, TX; [2]Neurosurg., Seton Brain and Spine Inst., Austin, TX

Abstract: Neural oscillations are typically analyzed using methods that assume the continuous presence of a stationary oscillatory signal with variable amplitude in canonical frequency bands (e.g. alpha band 8-12 Hz). However, numerous factors contribute to the presence and characteristics of neural oscillations, broadly including neuroanatomy, behavioral state, and recording equipment, which may lead to violations of these assumptions and poor spectral decomposition. We therefore developed the Oscillatory ReConstruction Algorithm ("ORCA"), a fully-automated method which provides optimized spectral decomposition of the amplitude, phase, and frequency of oscillations in adaptively identified bands. ORCA uses several novel band identification methods and then combines spectral estimates across bands to reconstruct the raw signal, resulting in quantitative assessment of the signal decomposition in terms of explained variance. Analyzing both human and rodent recordings, we found that ORCA provided significantly improved spectral decomposition compared to using canonical frequency bands. We further show that ORCA captures the predicted modulation of low-frequency "alpha" activity during human eye-closure, demonstrating the feasibility of using ORCA to track how oscillations are modulated by behavior. ORCA is thus a novel analytic tool that will allow researchers to investigate how non-stationary neural oscillations vary across behaviors, brain regions, individuals, and species.

Disclosures: A. Watrous: None. **R.J. Buchanan:** None.

Poster

252. Connectomics Analytics III

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 252.20/BB50

Topic: I.07. Data Analysis and Statistics

Support: DOE

Title: Neuromorphic algorithms enhance medical database analysis

Authors: *P. L. FOLLETT, G. D. KARPMAN, D. R. FOLLETT, P. J. HENDRICKSON;
Lewis Rhodes Labs, Concord, MA

Abstract: Automated analyses of electronic medical records (EMR) are underutilized and there remain substantial obstacles to generating high quality, clinically relevant information for clinicians. Consistent with other big data challenges, predictive analytics and machine learning algorithms fail to produce meaningful risk statements for rare health outcomes. Extracted data remains of low quality with poor predictability for individual events such as suicide. Data

analytics escalate time-consuming human involvement of professionals, remain of limited value in a clinical setting, and contribute to the frustrated response of many physicians to EMR demands. We have developed a complex set of neuromorphic algorithms that evaluate extracted features from unstructured data in a situational context that may have applied utility analyzing EMR databases. To be clinically valuable, sensitive screening methods of low incidence events such as suicide must confront the challenge of unmanageably high false-positive rates. For testing we generated a model population of 100,000 patients with physical and mental health diagnoses statistically representative of the VA database. We first analyzed the model database using traditional feature extraction techniques, generating alerts for patients with 2-15 times predicted risk for suicide. This renders 79,155 alerts (79.2%) for patients at increased risk, representing a clinically absurd majority of the database. However, initial analysis of the data using our set of neuromorphic behavioral rules that assess risk in context of co-morbidities decreases the number of patient alerts down to 5,323, a small fraction (6.7%) of the alerts produced by traditional methods. This behavioral analysis can be modified with more rules; a single additional constraint produced a more modest 766 patient alerts (0.8%) while identifying a higher risk group. Although this number still represents primarily false positives, additional constraints can be chosen that increase specificity further. Recognition of high-risk patients within the EMR currently requires time-consuming analysis of a vast database. Our proposed neuromorphic behavioral algorithms are capable of complex contextual feature extraction and are optimized for rapid evaluation of huge volumes of structured and unstructured data. This methodology could be useful for generating a clinically meaningful assessment of increased morbidity and mortality risk in patient populations.

Disclosures: **P.L. Follett:** None. **P.J. Hendrickson:** None. **D.R. Follett:** None. **G.D. Karpman:** None.

Poster

252. Connectomics Analytics III

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 252.21/BB51

Topic: I.02. Systems Biology and Bioinformatics

Support: FNRS - FRIA (Fonds pour la formation à la recherche dans l'industrie et dans l'agriculture)
ARC contract 14/19-060

Title: Assessing the structure-function relationship in the human brain: A control-theoretic perspective

Authors: *B. CHIÊM[1], F. CREVECOEUR[2], J.-C. DELVENNE[1];
[1]ICTEAM, [2]ICTEAM and Inst. of Neurosciences, Univ. Catholique de Louvain, Louvain-la-Neuve, Belgium

Abstract: Understanding the interplay between the structure and the function of the human brain is a central challenge in neuroscience. In particular, how a static anatomical architecture can support multiple activity patterns remains unclear. Here, we adopt a network approach combined with a control-theoretic framework to study this question.
We consider a structural brain network obtained from diffusion imaging and tractography, and a set of task-related functional brain networks based on pairwise correlations between BOLD time series. Our hypothesis is that these distinct functional networks emerge from different sets of input nodes driving the information flow across the structural wiring. To test this, we define a linear communication dynamics on the structural network. Then, we consider the controllability Gramians induced by different sets of input nodes. Indeed, the Gramian of a dynamical system reflects the similarity between the dynamical behaviour of the nodes, given a particular set of control inputs. Thus, we propose to select for each task a set of input nodes such that the corresponding Gramian is well correlated with the associated functional connectivity matrix.
For empirical validation, we employed MRI data from the Human Connectome Project (HCP) to build one structural brain network, one resting-state functional network and seven functional networks corresponding to the tasks included in the HCP protocols (emotion, gambling, language, motor, relational, social, working memory). We used a genetic algorithm to optimize the correlation between the controllability Gramian and each functional network, and identify candidate sets of input nodes for each task. Our results show that i) different input sets are related to different tasks, ii) some nodes form a "core" control structure including among others the anterior cingulate cortex, consistently with the literature [1], and iii) the correlation scores between the Gramians and the functional connectivity matrices are higher than those obtained with other methods (e.g. on average 22% above the recent results of Tipnis et al. [2]). For some tasks, we relate the identified input nodes to results previously reported in the literature on their potential role in the tasks.
Our contribution suggests that flexible and distributed control of information flow in the human brain can explain the emergence of various functional states from a single neural structure.
[1] Dosenbach, N.U., et al. PNAS, 104.26 (2007)
[2] Tipnis, U., et al. IEEE Tr. on Network Science and Engineering, (2018)

Disclosures: B. Chiêm: None. **F. Crevecoeur:** None. **J. Delvenne:** None.

Poster

252. Connectomics Analytics III

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 252.22/BB52

Topic: I.02. Systems Biology and Bioinformatics

Support: BMBF Grant 01GQ1302
BMBF Grant 01GQ1509

Title: How to spend less time on data management and more on the science: Open tools for efficient data organization, reproducible workflows, and collaboration

Authors: A. KOUTSOU[1], M. SONNTAG[1], J. VANEK[1], C. GARBERS[1], C. J. KELLNER[1], J. GREWE[2], *T. WACHTLER[1];
[1]Dept. of Biol. II, Ludwig-Maximilians-Universität München, Planegg-Martinsried, Germany;
[2]Eberhard-Karls-Universität Tübingen, Tübingen, Germany

Abstract: Scientists experience increasing demands on research data management, such as organizing data workflows in the lab, exchanging data for collaboration, or preparing data for publication. To reduce the workload associated with data management, we provide a suite of tools for key tasks including metadata collection, data organization, and data sharing.
With the odML[1] format, metadata can be collected from various sources in an experiment into a unified representation, which helps keeping information about experimental conditions available and enables automated project management tasks such as monitoring and analyzing experiment statistics[2], as well as conversion of metadata to other formats such as RDF[3] to utilize semantic web technologies.
To keep data and metadata organized, the NIX[4] data format enables effectively linking data and analysis results as well as the associated metadata. The format supports a wide range of data types, including electrophysiology and imaging data. NIX uses the odML metadata format and is integrated with the Neo[5] Python package for electrophysiology, enabling Neo users to store their data in a common open format.
The GIN[6] services help keeping track of data workflows and support collaborative data sharing. Using established versioning technology[7,8], GIN tracks changes and keeps previous versions accessible when datasets are updated. It makes it convenient to work from multiple workplaces while keeping data available and in sync. The service works with any kind of directory structure and file types, supporting the scientist's data organization while making it straightforward to share data within a lab or with off-site collaborators and to work on it together. Plugins offer extended features including configurable workflow automation or automated format validation with every recorded data change.
The tools presented are easy to use, can be combined with other approaches for reproducibility and data sharing[9,10,11], and enable efficient data management supporting the FAIR principles[12].
[1] RRID:SCR_001376, http://www.g-node.org/odml
[2] https://doi.org/10.3389/fninf.2016.00026
[3] https://www.w3.org/RDF/
[4] RRID:SCR_016196, http://www.g-node.org/nix
[5] RRID:SCR_000634, http://neuralensemble.org/neo
[6] RRID:SCR_015864, https://gin.g-node.org
[7] https://git-scm.com
[8] https://git-annex.branchable.com

[9] http://neuralensemble.org/sumatra
[10] http://bids.neuroimaging.io
[11] http://datalad.org
[12] https://doi.org/10.1038/sdata.2016.18

Disclosures: **A. Koutsou:** None. **M. Sonntag:** None. **J. Vanek:** None. **C. Garbers:** None. **C.J. Kellner:** None. **J. Grewe:** None. **T. Wachtler:** None.

Poster

252. Connectomics Analytics III

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 252.23/BB53

Topic: I.07. Data Analysis and Statistics

Support: IBS-R015-D1
NRF-2016H1A2A1907833
NRF-2016R1A2B4008545
IITP-2019-2018-0-01798

Title: Fully automated parcellation of the primary auditory cortex

Authors: *K. BYEON[1,4], B.-Y. PARK[1,4], H. S. LEE[5], S.-G. KIM[4,2], H. PARK[3,4];
[1]Dept. of Electrical and Computer Engin., Sungkyunkwan Univ., SUWON, Korea, Republic of; [2]Dept. of Biomed. Engin., Sungkyunkwan Univ., Suwon, Korea, Republic of; [3]Sch. of Electronic and Electrical Engin., Sungkyunkwan Univ., SUWON, Korea, Republic of; [4]Ctr. for Neurosci. Imaging Res., Inst. for Basic Sci., Suwon, Korea, Republic of; [5]Neurosci., Max Planck Inst. For Empirical Aesthetics, Frankfurt, Germany

Abstract: The human primary auditory cortex (PAC) occupies major parts of the Heschl's gyrus (HG). The macro-anatomical geometry of HG, however, is highly variable across individuals and provides only rough indication of the position and extent of PAC.In the current study, we aimed to construct an automated pipeline for defining PAC of the human auditory cortex. We combined myelin density and curvature information derived from magnetic resonance imaging (MRI) to better indicate the extent of PAC. We collected T1- and T2-weighted structural MRI from ten participants. The myelin density and curvature maps were calculated using the Human Connectome Project (HCP) minimal preprocessing pipeline. The procedure to define our PAC is as follows. We first started with the early auditory cortex defined by the HCP multi-modal parcellation atlas as the initial region of interest (ROI). Within this ROI, a Gaussian mixture model clustering was applied to the myelin density map (cluster size of two). This process yielded two subdivisions, highly myelinated center (putative core) and less myelinated surrounding (putative belt). The curvature information was then applied to adjust the putative

core area. To incorporate the prior knowledge that PAC corresponds close to HG, we drew a line in between HG and Heschl's sulcus. The two resulting subcompartments of the putative core were then dilated until they had spatial overlap. Finally, the subcompartment located on HG was selected as PAC. From ten participants, we calculated the mean surface area of PAC defined by our pipeline. The mean from the left hemisphere was 2,524 mm^3 (SD, 651 mm^3) and from the right hemisphere was 2,470 mm^3 (SD, 344 mm^3). The size of PAC area reported in previous studies range from 1,329 to 2,172 mm^3. Our method defined the extent of PAC greater than others because we intended to include all the primary architectonic areas, for example, the core area of non-human primates, which includes A1, R and RT. Here, we developed a fully automated pipeline to objectively define the human PAC using structural MRI data, and the outcomes were comparable to the previous studies suggesting the reliability of our pipeline.

Disclosures: K. Byeon: None. **B. Park:** None. **H.S. Lee:** None. **S. Kim:** None. **H. Park:** None.

Poster

252. Connectomics Analytics III

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 252.24/BB54

Topic: I.07. Data Analysis and Statistics

Support: IBS-R015-D1
NRF-2016H1A2A1907833
NRF-2016R1A2B4008545
IITP-2019-2018-0-01798

Title: Parcellation of the belt area in the human auditory cortex using multi-modal magnetic resonance imaging

Authors: *H. S. LEE[1], B.-Y. PARK[2], K. BYEON[3], H. PARK[3], D. POEPPEL[4];
[1]Max Planck Inst. For Empirical Aesthetics, Frankfurt am Main, Germany; [2]Sungkyunkwan Univ., SUWON-SI, Korea, Republic of; [3]SKKU, Suwon, Korea, Republic of; [4]Neurosci., Max-Planck-Institute For Empirical Aesthetics, Frankfurt, Germany

Abstract: Over a dozen subregions are defined in the non-human primate auditory cortex. The translation of these areas onto the human brain, however, remains unclear. Recently the Human Connectome Project (HCP), using magnetic resonance images, has successfully delineated 180 areas per hemisphere in humans which includes the auditory cortex roughly subdivided into five compartments. Further delineation and comparisons to non-human primate auditory cortex remain to be explored.
We have developed an automated pipeline defining the human primary auditory cortex (comparable to the core area in non-human primates) using myelin density and curvature

information from magnetic resonance images. In the current study, we aimed to further parcellate the surrounding belt area using resting-state functional magnetic resonance imaging (rs-fMRI) and verify the consistency of parcellation with diffusion tensor imaging. We started with the early auditory cortex defined by HCP multi-modal parcellation atlas as the initial region of interest. Within the ROI the core area was defined by our automated process and then excluded. The time series of all vertices in the remaining belt area that surround the core were extracted. Then three independent components were computed from the time series data. These components were identified in the anterior, medial, and posterior clusters within the belt area. Tractography was performed with the seed of each identified cluster using diffusion tensor imaging (deterministic) to assess the anatomical connections. The anterior subregion had connections to the prefrontal cortex including the primarily orbito- and medial-frontal and secondarily dorsolateral prefrontal cortices. The posterior subregion was connected to the visual cortex. The medial subregion showed connections both with the prefrontal and visual cortices. Our findings suggest that functionally distinct subregions within the belt area in humans show distinct anatomical connectivity patterns. These results might provide new insights on exploring the subregions of the belt area in the human auditory cortex.

Disclosures: H.S. Lee: None. **B. Park:** None. **K. Byeon:** None. **H. Park:** None. **D. Poeppel:** None.

Poster

252. Connectomics Analytics III

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 252.25/BB55

Topic: I.07. Data Analysis and Statistics

Support: CMU CIT Incubation Award
NSF WiFiUS program
CMU BrainHUB award

Title: Silence localization: First validation on real data

Authors: *A. CHAMANZAR, M. BEHRMANN, P. GROVER;
Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Objective and Rationale: We recently proposed an algorithm for localizing “silences” in the brain using non-invasive electroencephalography (EEG) signals, which compared favorably in simulations against classical source localization algorithms. “Silences” are regions of the brain without any electrical activity, which can be used to model many phenomena of clinical and neuroscientific interest, e.g., tissue affected by stroke, traumatic brain injuries, or lesions, and, in some cases, Cortical Spreading Depressions (CSDs). MRI and CT scans may be

difficult to acquire in emergency situations, and are also expensive and sometimes unusable if patients have metal implants. This paper tests this silence algorithm rigorously against real-data recorded from participants who have silences of varying sizes and locations in their brains. Methods and Results: We use a 128-electrode standard EEG grid to record scalp potentials while having the participants perform different visual tasks. Our key idea is to extract "variance reduction" of each source from the recorded dataset, which is obtained from the contribution of each source in the power of the recorded signals. This variance reduction is then used to localize the region of silence in the brain through an optimization framework. To test the algorithm, we recorded EEG signals on 3 male participants with silences in their brains: one with visual agnosia (male; 40 years, small lesion in the right ventral occipitotemporal cortex), and 2 with resections (males, both 12 years, large resected regions in the right occipitotemporal for one, and the left temporal lobe for the other). Structural MRI was used as the ground truth. Our algorithm localized these silences to a good degree of accuracy, as shown in the attached Figure. Conclusions: This study validates our novel approach for non-invasive localization of silenced regions in the brain on 3 patients with different types/sizes of silences. Further validation is needed, including on patients with reduced activity instead of complete silence.

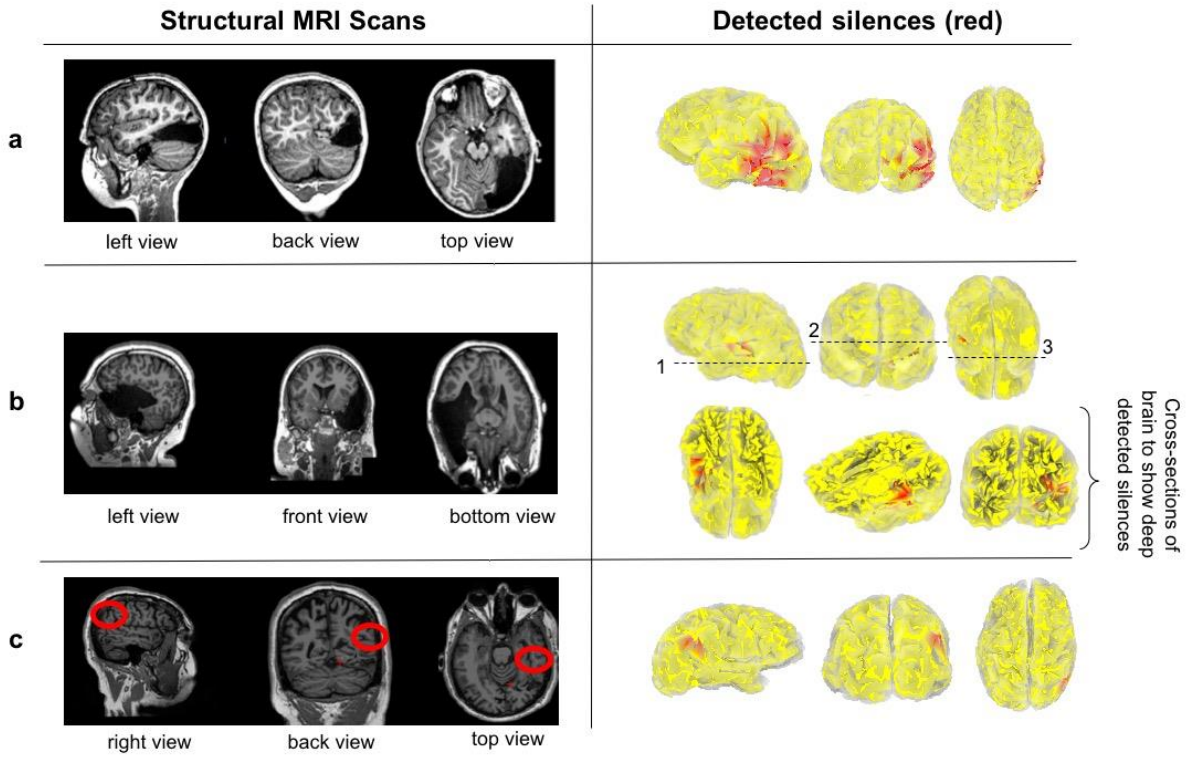


Fig. Validation of silence localization based on real EEG signals. The left column shows the structural MRI scans of three subjects with different sizes/types of silences in the brain. The right column shows the detected silenced regions in the corresponding subjects: (a) a 12 year old male with right occipitotemporal lobectomy, (b) a 12 year old male with left temporal lobectomy, and (c) a 40 year old male with a small lesion in the right ventral occipitotemporal cortex.

Disclosures: **A. Chamanzar:** None. **M. Behrmann:** None. **P. Grover:** None.

Poster

252. Connectomics Analytics III

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 252.26/BB56

Topic: I.07. Data Analysis and Statistics

Title: Machine learning distinguishes Parkinson's disease from epilepsy on MRI

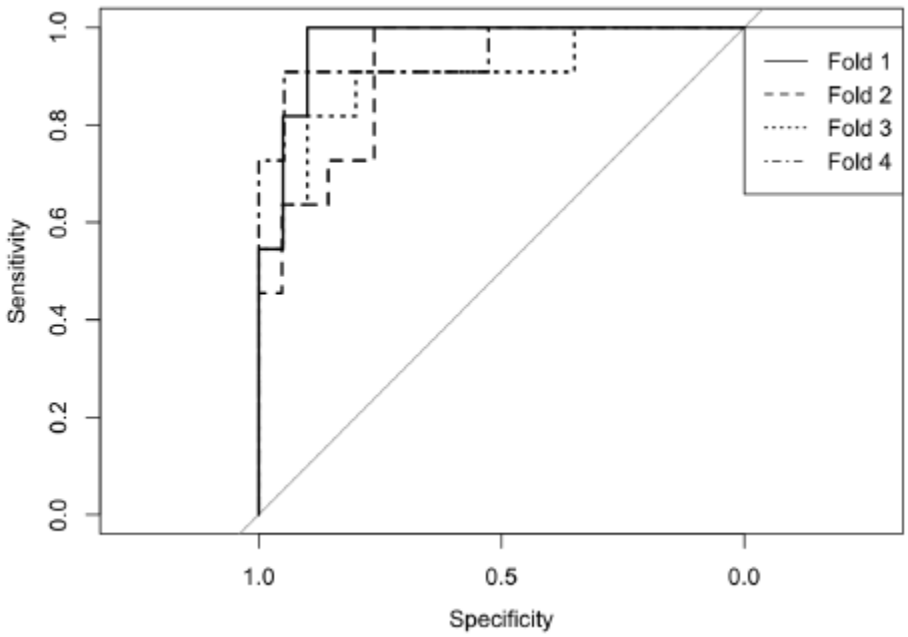
Authors: *R. O'DWYER[1], B. COZZI[2], D. GARIBAY[2], K. OGDEN[3], J. G. GOLDMAN[4], T. R. STOUB[5];
[1]Rush Epilepsy Ctr., [2]Rush Univ. Med. Ctr., Chicago, IL; [3]Radiology, Upstate Med. Center, SUNY, Syracuse, NY; [4]Northwestern Univ., Chicago, IL; [5]Neurolog. Sci., Rush Univ., Chicago, IL

Abstract: Over the past 15 years researchers from many disciplines have demonstrated the ability of machine learning algorithms to accurately detect subtle differences between patients to make disease classifications. Radiomics uses high-throughput extraction of quantitative features from medical images and then employs machine learning algorithms, to uncover disease characteristics that cannot be seen by the naked eye. These machine learning algorithms perform particularly well on neuroimaging applications and have been applied to detect multiple sclerosis, and seizures. 1.5T MRimaging was obtained from 162 subjects from five groups(Parkinson’s Disease [PD]; Temporal Lobe Epilepsy [TLE]; Young controls and Older controls). Using FreeSurfer, five regions of interest (ROI) were parcellated (Hippocampus, Amygdala, Cingulate gyrus, Entorhinal cortex & Thalamus). These ROIs are implicated in TLE, PD & undergo changes with aging. Using ITK Toolbox, 53 radiomic features were extracted from each ROI & analysed using a random forest machine learning algorithm. 4-fold cross validation was performed. The Receiver Operating Curve (ROC) for the classification of the subjects for each Fold is shown in Figure 1, & had an Area Under Curve (AUC) of 0.93. This demonstrates that machine learning algorithms can accurately classify different neurological disorders and different aged controls with high sensitivity and specificity. This proof of concept study highlights the potential for this novel methodology to be further developed into radiomic signatures for various neurological disorders that could be used as diagnostic, non-invasive and cost-effective biomarkers.

Table 1

Group	n	Mean Age (range)	Female/Male
Aged Controls	34	77yrs(65-89)	23/11
Young Controls	53	28yrs (20-56)	25/28

Temporal Lobe Epilepsy	49	36yrs(19-60)	26/23
Parkinson's Disease	25	73yrs(70-76)	6/19
Subject Demographics			



Disclosures: **R. O'Dwyer:** None. **B. Cozzi:** None. **D. Garibay:** None. **K. Ogden:** None. **J.G. Goldman:** None. **T.R. Stoub:** None.

Poster

252. Connectomics Analytics III

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 252.27/BB57

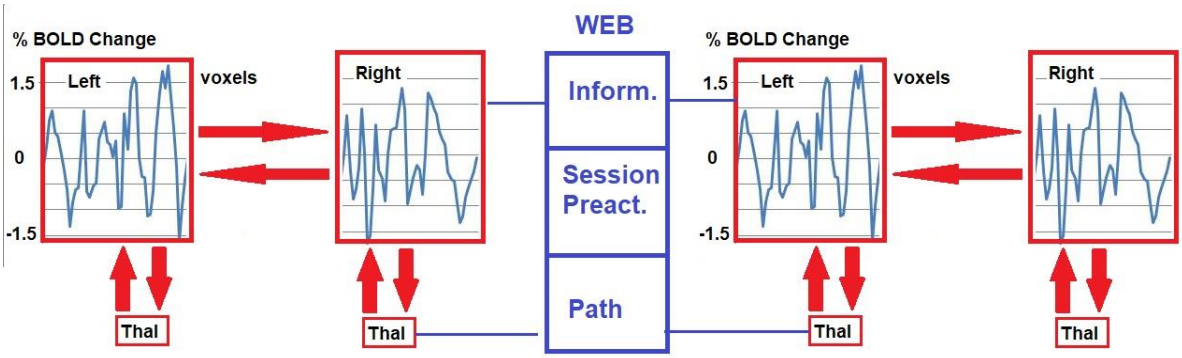
Topic: I.07. Data Analysis and Statistics

Support: Personal Support only for JFGM. IGN pending.

Title: A statistical/fuzzy metaphor for fMRI/EEG oscillations: are EM-clouds controlled and functionally connected by the thalamus?

Authors: *J. F. GOMEZ-MOLINA;
Intl. Group of Neurosci. (IGN), Medellin, Colombia

Abstract: MRI/EEG-techniques open the door to study new ElectroMagnetic (EM) phenomena in brain tissue beyond the reach of classical, invasive electrophysiology, like delicate neurocircuits involving ephaptic links (Gomez, Perry, Ricoy et 2018/9, Proposals Social Issue Roundtable, SfN). Spatial correlations along paths and networks in susceptibility, polarizability and T2* inhomogeneities require probabilistic approaches. Invasive techniques based on classic physic are reaching the limit. We propose the metaphor of EM-clouds to inspire a new philosophical foundation of statistics based on complex numbers for probabilities beyond the conventional Bayesian and frequentists interpretations. METHODS. Agorithms. Python. Computationally generated signals. RESULTS. We worked on algorithms to extract intervals of fMRI signals defined between points in which the n-derivative of %BOLD is zero. CONCLUSIONS. 1. Neuroscience will be soon without a general consensus about interpretation of EM-phenomena, as it is now modern physics. Neuroengineering will be an intuitive set of rules to guide designs in the new applications. 2. EM-clouds are metaphors for EM-probabilistic activity inside the brain. They may brings all the theoretical controversies and unsolved issues of quantum mechanics to EEG/fMRI and neuroscience, but also the incredible engineering designs and applications of EEG-WEB Protocols (OSI-type) with artificial intelligence systems (Gomez JF 2019, ""Protocolo EEG-WEB 3.0 de acople delicado, epáptico, egoente y con libre (de)sincronización" https://www.otraparte.org/actividades/ciencia/dia-de-la-ciencia-ciudadana-2019.html Citizen-Science Conferences, Otraparte/BPP Medellin).



Disclosures: **J.F. Gomez-Molina:** None.

Poster

252. Connectomics Analytics III

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 252.28/BB58

Topic: I.02. Systems Biology and Bioinformatics

Title: Dimensionality reduction in systems analysis of a drug vs. complex phenotype relationship: Lithium, BDNF, and neural disease

Authors: T. MANGETI GONCALVES[1], ***E. JAKOBSSON**[2];
[1]Washington Univ., St. Louis, MO; [2]Univ. of Illinois At Urbana-Champaign, Urbana, IL

Abstract: The relationship between a drug and a complex multigenic phenotype is inherently difficult to unravel. In this paper we show one approach to reducing the dimensionality of this problem by focusing on an exemplary problem: the relationships among lithium, BDNF, and neural disease. Reduced serum levels of brain-derived neurotrophic factor (BDNF) have been found associated with depression, bipolar disorder, and dementia. Lithium has been found to elevate BDNF. Understanding these associations may help to elucidate mechanisms underlying the effectiveness of lithium against depression and bipolar disorder and suggest avenues for prevention and treatment of dementia. However, it is expected that these associations will not be simple. Lithium is known to inhibit seventeen different phosphotransferases, which among them interact with hundreds of substrates. BDNF is central to the neurotrophin signaling pathway and involved in several others. In order to reduce the dimensionality of the problem of understanding the lithium-BDNF association, we interrogate databases of protein-protein interactions and disease-relevant pathways to construct subnetworks comprised of the intersection of 1) the BDNF interactome, 2) the interactomes of the lithium-sensitive phosphotransferases, and 3) pathways thought to be relevant to depression, bipolar disorder, and dementia. In this presentation we display the subnetworks and discuss activity in the networks likely to mediate the lithium-BDNF connection, with possible relevance to neural disease. For the particular case of bipolar disorder, the process produces a subnetwork of 50 interacting proteins common to the BDNF interactome, the putative bipolar network, and the lithium interactome. It includes two lithium-sensitive phosphotransferases, GSK3B and ACDY2. We hypothesize that this network contains the key elements of the mechanism by which lithium ameliorates bipolar disorder, and that the distinction between lithium responders and nonresponders resides in variations in either the components of this network or regulatory elements targeting these components. We suggest that this approach may elucidate other drug-response relationships, by augmenting the concept of a molecular drug target with the concept that a drug may target a definable network related to one or more phenotypes.

Disclosures: T. Mangeti Goncalves: None. **E. Jakobsson:** None.

Poster

253. Optogenetics I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 253.01/BB59

Topic: I.08. Methods to Modulate Neural Activity

Support: NIDA/IRP

Title: Distinct roles of VTA glutamate, GABA and glutamate-GABA neurons in motivated behavior

Authors: D. H. ROOT[1], D. J. BARKER[2], D. J. ESTRIN[1], J. A. MIRANDA-BARRIENTOS[3], B. LIU[4], S. ZHANG[5], F. VAUTIER[6], C. RAMAKRISHNAN[7], Y. S. KIM[9], L. E. FENNO[8], K. DEISSEROTH[7], *M. F. MORALES[10];
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Abstract: Dopamine neurons of the ventral tegmental area (VTA) have been implicated in different aspects of reward and aversion-related motivated behavior. In addition to dopamine neurons, the VTA has GABA and glutamate neurons that have been shown to play roles in reward and aversion. We recently discovered that the VTA contains neurons that co-release glutamate and GABA from different pools of synaptic vesicles. These glutamate-GABA neurons co-express transporters for the vesicular uptake of either glutamate (vesicular glutamate transporter type 2, VGluT2) or GABA (vesicular GABA transporter, VGAT). By VTA cellular mapping, we had found that VGluT2-VGAT neurons are intermixed with neurons that express solely VGluT2 (VGluT2-only neurons) or solely VGAT (VGAT-only neurons). Towards determining the roles of VTA VGluT2-only, VGAT-only and VGluT2-VGAT neurons in motivated behavior, we crossed*Vglut2-Cre* mice with *Vgat-FlpO* mice and in the VTA of these dual Cre/FlpO transgenic mice injected newly developed INTRSECT viral vectors to selectively target each class of neurons. By anatomical and electrophysiological analyses, we first established the selective recombination of each targeted class of neurons within the VTA of viral injected Cre/FlpO mice. In a follow up study, we injected in the VTA of Cre/FlpO mice newly developed INTRSECT GCaMP6m viral vectors, and after verification of the specific expression of GCaMP6m within the targeted class of neurons, used these mice to detect calcium transients (as an indicator of neuronal activity) in response to reward, punishment, or the presentation of learned cues predicting the delivery of these stimuli. Regarding reward processing, we found that while all three of the examined classes of VTA neurons responded to the presence of a sucrose reward, the VGluT2-only neurons, (but not VGAT-only or VGAT-VGluT2 neurons) responded to reward-predictive cues and discriminated between cues that did or did not predict the delivery of reward. Regarding aversive processing, we found that while all three classes of VTA neurons responded to the presentation of an aversive footshock, both VGluT2-only and VGAT-only neurons (but not VGluT2-VGAT neurons) responded to cues predicting footshock delivery. These findings indicate that within the total population of VTA VGluT2 and VGAT neurons, there are selective classes of neurons that have both overlapping as well as unique roles in signaling specific aspects of motivated behavior.

Disclosures: **D.H. Root:** None. **D.J. Barker:** None. **D.J. Estrin:** None. **J.A. Miranda-Barrientos:** None. **B. Liu:** None. **S. Zhang:** None. **F. Vautier:** None. **C. Ramakrishnan:** None. **Y.S. Kim:** None. **L.E. Fenno:** None. **K. Deisseroth:** None. **M.F. Morales:** None.

Poster

253. Optogenetics I

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Program #/Poster #: 253.02/BB60

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH grant R01 MH117128
NIH grant R01 DA038966

Title: Dopamine-glutamate neurons facilitate behavioral switching under circumstances of altered cue-reinforcer contingencies

Authors: *S. MINGOTE[1,2], S. ZTAOU[2], A. AMSELLEM[2], A. KEMPF[2], S. OH[2], E. O'LEARY[3], R. LOGAN[3], F. WEISEL[4], Z. FREYBERG[3], Y. KIM[5], L. E. FENNO[5], C. RAMAKRISHNAN[5], K. DEISSEROTH[5], S. RAYPORT[2];
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Abstract: Dopamine (DA) neurons in the ventral tegmental area (VTA) capable of glutamate (GLU) cotransmission project to the nucleus accumbens (NAc) shell (Mingote et al., *J Neuroscience*, 2015). In the NAc shell, they make strong GLUergic connections to cholinergic interneurons, and can control their activity (Chuhma et al., *Neuron*, 2014). Decreasing GLU cotransmission by reducing the expression of glutaminase (gene *Gls1*) in DA neurons selectively interferes with the ability of the neurons to drive cholinergic interneurons to burst fire. Behaviorally, mice with reduced *Gls1* in their DA neurons show potentiated latent inhibition (Mingote et al., *eLife*, 2017). In this paradigm, mice initially receive multiple presentations of a tone, which in a later session are paired with a mild shock, so that animals have to switch from a tone-nothing to a tone-shock association. The delay in switching characterizes latent inhibition. We hypothesized that the DA neuron GLU signal facilitates behavioral switching. To test this further, we used the INTRSECT combinatoric viral strategy (Fenno et al., *Nature Met*h, 2014) that allows for selective expression of channelrhodopsin-EYFP in either DA-GLU or DA-only neurons. The specificity of the viruses was verified using fluorescence activated cell sorting combined with mRNA sequencing and immunohistochemistry. Imaging of fluorescently labeled terminals in the NAc revealed that DA neurons projecting to the dorsal NAc medial Shell are exclusively DA-GLU neurons; DA-GLU neurons project strongly to the NAc lateral Shell and sparsely to the NAc Core. Stimulating DA-GLU neurons in the VTA *in vivo* disrupted latent inhibition, thus facilitating the switching from responding to a tone-nothing to a tone-shock association. These data suggest that DA-GLU neurons projecting to the NAc shell play a crucial

role in adjusting behavioral responses in situations of greater uncertainty, when cue-reinforcer contingencies are altered.

Disclosures: **S. Mingote:** None. **S. Ztaou:** None. **A. Amsellem:** None. **A. Kempf:** None. **S. Oh:** None. **E. O'Leary:** None. **R. Logan:** None. **F. Weisel:** None. **Y. Kim:** None. **L.E. Fenno:** None. **C. Ramakrishnan:** None. **K. Deisseroth:** None. **S. Rayport:** None. **Z. Freyberg:** None.

Poster

253. Optogenetics I

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Program #/Poster #: 253.03/BB61

Topic: I.08. Methods to Modulate Neural Activity

Support: Walter V. and Idun Berry Postdoctoral Fellowship
Gatsby Foundation
HHMI

Title: The role of fronto-striatal connectivity in value-based decision-making

Authors: ***F. GORE**, C. RAMAKRISHNAN, R. C. MALENKA, K. DEISSEROTH; Stanford Univ., Stanford, CA

Abstract: Organisms exist in complicated environments that contain many sensory stimuli with differing motivational values. Animals must evaluate these different stimuli and decide which to pursue. Evidence has accumulated implicating the orbitofrontal cortex (OFC) in this process; however how orbitofrontal neurons engage downstream circuits to compute and execute value-based choice remains largely unknown. Here, we use a combination of behavioral, anatomical, and optogenetic approaches to examine the role of OFC projections to distinct brain areas in value-based decision-making. First, by developing a novel value-based decision-making paradigm for rats, we have demonstrated that rats are capable of making appropriate value-based decisions; animals consistently choose more valuable options (fraction correct=82.56±0.64%, n=19), and choice latency is proportional to trial difficulty (r=0.29, p=0.03). Next, by rendering whole rat brains transparent, we have revealed robust projections from the orbitofrontal cortex to several brain areas, including the dorsal striatum. Finally, by optogenetically inhibiting the activity of OFC axon terminals in different brain areas, we have demonstrated that OFC projections specifically to the dorsal striatum are critically required for optimal value-based decision-making (fraction correct: uninhibited=83.42±1.86%, inhibited=68.11±1.34%, $p<0.01$, n=6), but not other goal-directed motivated behaviors. Taken together, these data illuminate a central role for fronto-striatal connectivity in value-based decision-making.

Disclosures: **F. Gore:** None. **C. Ramakrishnan:** None. **R.C. Malenka:** None. **K. Deisseroth:** None.

Poster

253. Optogenetics I

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Topic: I.08. Methods to Modulate Neural Activity

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Brain Research Foundation Fay/Frank Seed Grant
Citizens United for Research in Epilepsy (CURE) Taking Flight Award
Beckman Institute Postdoctoral Fellowship
Beckman Institute Undergraduate Fellowship

Title: Optogenetic stimulation of astrocytes alters synaptic transmission in hippocampal CA1 in an opsin- and stimulation-specific manner

Authors: *C. D. COURTNEY[1], C. SOBIESKI[2,3], N. M. WOJNOWSKI[2,3], C. RAMAKRISHNAN[4], R. A. DEFAZIO[5], K. DEISSEROTH[4], C. A. CHRISTIAN[1,2,3];
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Abstract: Astrocytes can detect synaptic signaling through G protein-coupled neurotransmitter receptors, but the subsequent effects of this response on synaptic function are less understood, as there has been a limited availability of tools that selectively activate G protein signaling pathways in astrocytes. While there is growing evidence that astrocytes actively modulate synaptic excitation, the extent to which astrocytes modulate inhibition remains unknown. Here, we used optogenetics to investigate opsin- and stimulation-specific activation of astrocytes and subsequent modulation of synaptic transmission to hippocampal CA1 pyramidal cells. C57BL/6J mice were injected in dorsal CA1 with adeno-associated virus (AAV8) to express either light-sensitive Gq-linked alpha1 adrenergic receptor (optoα1), channelrhodopsin (ChR2), or control GFP, all under the astrocyte promoter GFAP. Successful targeting of opsin/GFP to astrocytes was confirmed by histology. At least one month after virus injection, we made whole-cell patch clamp recordings of spontaneous inhibitory or excitatory postsynaptic currents (sIPSCs/sEPSCs) in pyramidal cells in acute slices. Blue laser light (473 nm) was delivered to the slice via optical fiber under two different stimulation paradigms: 0.5 Hz (1 s pulses at successive 1, 5, and 10 mW intensities, 90 s per intensity) or 20 Hz (45 ms pulses, 5 mW intensity, 5 min). With 0.5 Hz stimulation in either optoα1- or GFP-expressing slices, no differences in sIPSC or sEPSC frequency/kinetics were seen (n=10 cells/group). With 20 Hz stimulation, however, cells from

slices expressing optoα1 displayed a decrease in sIPSC amplitude compared to baseline (n=16 cells; p<0.001, KS test) and compared to control GFP (n=8 cells; p<0.0001, KS test), as well as a trend for an increase in sIPSC frequency compared to baseline (p=0.07, paired t-test). Under both stimulation paradigms, astrocytic ChR2 activation drove a potent increase in both sEPSC and sIPSC frequency (0.5 Hz n=7-9 cells/group; 20 Hz n=4-6 cells/group) immediately following light exposure. The EPSC frequency effect was blocked by tetrodotoxin (n=9 cells). These findings suggest that the nature and mechanism of astrocytic stimulation may play a critical role in the modulation of synaptic transmission in CA1.

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Poster

253. Optogenetics I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 253.05/BB63

Topic: I.08. Methods to Modulate Neural Activity

Support: Helen Hay Whitney Foundation (MLB & ASA)
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NIMH K99 MH112840 (MLB)
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NIMH (KD)
NIDA (KD)

Title: A convergent hypothalamus-brainstem circuit for rapid avoidance of homeostatic threats

Authors: *M. LOVETT-BARRON[1], R. CHEN[1], S. BRADBURY[1], A. S. ANDALMAN[1], M. WAGLE[2], S. GUO[3], K. DEISSEROTH[4];
[1]Stanford Univ., Stanford, CA; [2]Univ. of California San Francisco, San Francisco, CA; [3]Univ. California - San Francisco, San Francisco, CA; [4]Bioengineering, Stanford University, HHMI, Stanford, CA

Abstract: The hypothalamus coordinates behavioral states that are manifested over long timescales, such as homeostatic adaptations to environmental stressors. As in other vertebrates, when zebrafish encounter environmental stressors (increased heat, salinity, acidity, etc.), the hypothalamus initiates the release of stress hormones over minutes, allowing for physiological adaptations. Zebrafish also execute fast evasive behaviors at the onset of these stressors, in order to prevent such need states. It is unknown whether these faster responses also involve the hypothalamus.

Using controlled stimulus delivery to tethered larval zebrafish, we found that the onset of stressors (heat, +7C; salinity, +50mM NaCl; acidity, +0.1mM HCl) increased the rate of turning behavior ($p<0.05$ within 20s of stimulus onset). Brain-wide two-photon calcium imaging demonstrated that increases in heat, salinity, or acidity recruits neurons distributed throughout the forebrain, hindbrain, and neurosecretory hypothalamus - regions also implicated in slower homeostatic responses.
The neurosecretory preoptic hypothalamus (nPO) is composed of many peptidergic cell types. To image these neurons, we elaborated upon our MultiMAP method to perform cellular-level registration of neural activity to gene expression, visualized through multiple rounds of multi-color fluorescent *in situ* hybridization (for *avp*, *oxt*, *crf*, *sst*, *npy*, and *vip*). Rather than cellular specificity, we found that functional cell classes span multiple transmitter-defined cell types. Furthermore, optogenetic activation of either *oxt*, *avp*, or *crf* neurons could induce turning behavior ($p<0.01$, vs ChR2- controls), but conditional ablation with NTR did not reduce threat-induced avoidance ($p>0.1$). However, broader ablation of *otpba* neurons in the nPO suppressed turning responses to heat, salinity, and acidity ($p<0.01$). These data suggest that multiple peptidergic cell types in the nPO may play common roles in threat avoidance. Anatomical tracing indicates these neurons converge on common action-selection circuits in the brainstem, linking neuroendocrine cell types to locomotion.
Animals can respond to homeostatic stressors with fast avoidance or slow adaptation. Here we show that fast responses recruit some of the same hypothalamic circuitry used for slow adaptation, but with a mechanism that drives rapid escape through brainstem circuits. The molecular and functional diversity of the vertebrate hypothalamus allows this structure to flexibly regulate survival behaviors across multiple timescales.

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Poster

253. Optogenetics I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 253.06/BB64

Topic: I.08. Methods to Modulate Neural Activity

Title: Minimally-invasive optogenetics in mice and non-human primates with a new high-sensitivity opsin

Authors: *X. GONG[1,2], D. MENDOZA-HALLIDAY[1], J. T. TING[3,4,1], T. KAISER[1], X. SUN[1,5], A. BASTOS[1], R. D. WIMMER[1], C. WU[1], B. BARAK[1], K. DEISSEROTH[6], E. K. MILLER[1], M. M. HALASSA[1], G. BI[2], R. DESIMONE[1], G. FENG[1];
[1]MIT, Cambridge, MA; [2]Univ. of Sci. and Technol. of China, Hefei, China; [3]Human Cell Types,

Allen Inst. for Brain Sci., Seattle, WA; [4]Univ. of Washington, Dept. of Physiol. and Biophysics, Seattle, WA; [5]Zhejiang Univerisity, Hangzhou, China; [6]Bioengin & Psych, Stanford Univ. Dept. of Psychology, Stanford, CA

Abstract: Optogenetics is a widely employed technique for perturbation of neuronal activity, and is recently being considered as a potential treatment of neurological disorders. Currently, one of its major downsides is the need for invasive implantation of optical fibers. While an ideal solution to this problem would be the use of external optical stimulation, the levels of light power that typically reach the target brain regions are too low to activate currently available opsins. To overcome this challenge, we designed a minimally-invasive optogenetic method by engineering SOUL, a new step-function opsin with ultra light sensitivity. We show that SOUL's photocurrent is three times that of its parental opsin - SSFO. We further show that SOUL can activate and subsequently deactivate neurons located in deep mouse brain regions by transcranial optical stimulation, and that such effect is strong enough to disrupt and restore feeding behaviors in SOUL knock-in mice. Moreover, SOUL allowed us to reversibly modulate neuronal spiking and induce local field potential oscillations in macaque cortex with optical stimulation from outside the brain and the dura. This provides a means to activate large primate cortical areas. Collectively, our results demonstrate the use of SOUL in a novel method for minimally-invasive optogenetic stimulation in mice and macaques that allows long-lasting, large-scale neuronal activation while avoiding the damage and risks associated with optical fiber implantation and exposure of the brain surface. This method may facilitate the development of minimally-invasive treatments for neurological disorders.

Disclosures: X. Gong: None. **D. Mendoza-Halliday:** None. **J.T. Ting:** None. **T. Kaiser:** None. **X. Sun:** None. **A. Bastos:** None. **R.D. Wimmer:** None. **C. Wu:** None. **B. Barak:** None. **M.M. Halassa:** None. **E.K. Miller:** None. **K. Deisseroth:** None. **G. Bi:** None. **R. Desimone:** None. **G. Feng:** None.

Poster

253. Optogenetics I

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Program #/Poster #: 253.07/BB65

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant 5R37MH075957-12

Title: INTRSECT 2.0: Enabling viral targeting of multiply-defined populations of neurons

Authors: *L. E. FENNO[1], C. RAMAKRISHNAN[2], Y. KIM[2], K. E. EVANS[3], S. VESUNA[2], M. INOUE[2], M. K. LO[2], K. CHEUNG[2], N. PICHAMOORTHY[2], K. DEISSEROTH[1];

[1]Bioengineering, Psychiatry, [2]Bioengineering, Stanford Univ., Stanford, CA; [3]Harvard Med. Sch., Cambridge, MA

Abstract: The development of molecular tools has dramatically advanced systems neuroscience research. These include tools that allow researchers to control populations of neurons with millisecond resolution, monitor the activity of populations of neurons, and selectively infect neurons with modified viruses for circuit tracing. Use of these genetically-based approaches is limited by the ability of researchers to target them to specific populations of neurons. We previously published a proof-of-concept technique that allows molecular tools to be expressed in neurons based on multiple genetic and/or circuit architecture parameters, termed INTRSECT (for 'intronic recombinase sites enabling combinatorial targeting'). This approach relies on the combination of highly engineered viruses and patterns of recombinases. The initial complement of INTRSECT viruses has been used to describe the projection patterns of multiply-defined neuron subtypes and understand the contribution of defined neuron populations to a wide range of behaviors. Considering the success to date and potential to impact neuroscience widely, we have worked to further expand the INTRSECT approach through four parallel engineering approaches: 1) expansion of the INTRSECT toolbox to include all commonly used molecular reagents for neuroscience through an engineering and validation pipeline, bringing the total number of validated tools to 42; 2) Improvement of the Flp-dependent components of the INTRSECT backbone via *in vitro* variant screening with ~20% improvement in Flp efficacy; 3) development of an inexpensive device to monitor viral expression *in vivo* and describe viral expression kinetics over 6 weeks of expression; and 4) further expansion of the targeting resolution of INTRSECT to triple-recombinase (Cre, Flp, VCre) dependence and validation of this approach *in vivo*. As the suite of molecular tools continues to expand, techniques such as INTRSECT are an integral, but currently absent, element required to continue driving basic neuroscience research forward.

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Poster

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Topic: I.08. Methods to Modulate Neural Activity

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NARSAD Young Investigator Grant
Amazon AWS Grant

NIMH
NIDA
DARPA
NSF

Title: Neuronal dynamics regulating brain and behavioral state transitions

Authors: *A. S. ANDALMAN[1], V. M. BURNS[1], M. LOVETT-BARRON[1], M. BROXTON[1], B. POOLE[1], S. J. YANG[1], L. GROSENICK[1], T. N. LERNER[1], R. CHEN[1], T. BENSTER[1], P. MOURRAIN[1], M. LEVOY[1], K. RAJAN[2], K. DEISSEROTH[3];
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Abstract: The modulation of behavioral based on the success or failure of past action patterns is a natural and adaptive process, through which innate behavioral predilections set by genetics and development can become tuned to the statistics of environmental challenges and opportunities in the present. Such behavioral state modulation can also become maladaptive (for example, in certain symptoms of depression). These behavioral state changes have been studied extensively in rodent behavioral-challenge assays, in which initial active coping (involving vigorous motor response to an inescapable challenge) is observed to transition, after repeated failures, to a passive-coping strategy. A complete brain-wide and causal perspective on the nature of these important states at the cellular level has been elusive, due in part to barriers associated with achieving full access for imaging and control across the mammalian brain during complex behavioral state transitions. Here, we have developed a paradigm for behavioral state transitioning from active- to passive- coping in the larval zebrafish ($n=33; p<0.001$ Student's t-test) and used brain-wide imaging to perform unbiased global exploration for involved circuit elements. We found that this important state transition is specifically manifested by the recruitment of individual habenular neurons into an activated ensemble, that temporally tiled the duration of the stressor. The resulting ramping of neural activity exhibited several properties of interest: 1) was restricted to the habenula across the entire brain (while other regions such as the serotonergic raphe nucleus display decreasing, synchronous, and saturating changes); 2) had the effect of reporting on the important duration statistic of the behavioral stressor; 3) could be bi-directionally modulated, including by prior stressful experience; and 4) significantly accounted for inter-individual behavioral variability in the state transition ($n=20$; $r=-.56$, $p<.005$). We used targeted optogenetic stimulation combined with brain-wide imaging to demonstrate that habenula activation was sufficient to modulate passivity behavioral responses, as well as to selectively alter activity in other brain regions (including the raphe) in a pattern similar to that naturally recruited by the inescapable challenge. Together, these results have identified the progressive recruitment of habenular neurons as a mechanism by which important parameters of ongoing experience are encoded, and in the broader context of vertebrate evolution may reveal an ancestral process by which vertebrate brain-wide activity states and behavioral state transitions can be implemented.

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Poster

253. Optogenetics I

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Program #/Poster #: 253.09/BB67

Topic: I.01. Molecular/ Biochemical/ and Genetic Techniques

Support: DA044696
2PN2EY018241

Title: Genetically-targeted optical control of endogenous G protein-coupled receptors

Authors: *P. C. DONTHAMSETTI[1], N. WINTER[3], S. LAMMEL[2], D. TRAUNER[4], E. ISACOFF[1];
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Abstract: G protein-coupled receptors (GPCRs) play essential neuromodulatory roles in the brain. However, their spatial organization and temporal profiles of activation are highly complex, making it difficult to interrogate individual receptors with sufficient precision. State-of-the-art approaches that are used to control GPCR signaling either (i) require engineered and possibly non-physiological proteins (DREADDs, optoXRs, PORTL-gated receptors), or (ii) control endogenous receptors in a manner that cannot be turned on and off (t-toxins, DARTs). Using a combination of chemistry, biology, and light, we devised an approach that can be used to control endogenous GPCRs with molecular-, cell type-, and spatio-temporal precision. We targeted two important and structurally distinct classes of neuromodulatory GPCRs: metabotropic glutamate receptors (mGluRs) and dopamine receptors (DARs). These receptors were rapidly, reversibly, and selectively activated with photoswitchable ligands tethered to a genetically targeted-plasma membrane anchor (membrane anchored Photoswitchable Orthogonal Remotely Tethered Ligands; maPORTLs). Our findings provide a template for controlling endogenous GPCRs as well as other membrane proteins with unprecedented precision.

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Poster

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Program #/Poster #: 253.10/BB68

Topic: I.08. Methods to Modulate Neural Activity

Support: DA039650
DA034681
MH114990
NS061788
DA042514

Title: Blue light induced gene expression alterations in cultured neurons are the result of phototoxic interactions with neuronal culture media

Authors: *C. G. DUKE, K. E. SAVELL, R. A. PHILLIPS, J. J. DAY;
Neurobio., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Blue waveform light is used as an optical actuator in numerous optogenetic technologies employed in neuronal systems. However, the potential side effects of blue waveform light in neurons has not been thoroughly explored, and recent reports suggest that neuronal exposure to blue light can induce transcriptional alterations *in vitro* and *in vivo*. Here, we examined the effects of blue waveform light in cultured primary rat cortical neurons. Exposure to blue light (470nm), resulted in upregulation of several immediate early genes (IEG) traditionally used as markers of neuronal activity, including *Fos* and *Fosb*, but did not alter the expression of the circadian clock genes *Bmal1, Cry1, Cry2, Clock, or Per2*. IEG expression was induced with as little as 1 hour of 5% duty cycle light exposure. Elevated levels of blue light exposure induced a loss of cell viability *in vitro*, suggestive of overt phototoxicity. Notably, blue light induced changes in gene expression were prevented when neurons were cultured in a photoinert media supplemented with a photostable neuronal supplement instead of commonly utilized neuronal culture media and supplements. However, culture media exposed to blue light prior to adding it to cells failed to induce a detectable IEG response over non-light exposed media. Together, these findings suggest that the blue light induced gene expression alterations observed *in vitro* stem from a phototoxic interaction between commonly used media and neurons, and offer a solution to prevent this toxicity when using photoactivatable technology *in vitro*.

Disclosures: C.G. Duke: None. **K.E. Savell:** None. **R.A. Phillips:** None. **J.J. Day:** None.

Poster

253. Optogenetics I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 253.11/BB69

Topic: I.08. Methods to Modulate Neural Activity

Support: National Science Foundation (01A-1632881)

Title: Effects of X-irradiation on neuronal and circuit function

Authors: *A. F. BARTLEY[1], J. A. BARNES[2], D. N. FRENCH[3], T. R. TOTSCH[3], G. M. GRAY[3], L. L. MCMAHON[2], L. E. DOBRUNZ[1];
[1]Neurobio., [2]Cell, Developmental, and Integrative Biol., [3]Chem., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Optogenetics is a widely used technique in neuroscience. However for *in vivo* studies, the invasive nature of current methods for light delivery into the brain can cause damage to brain regions of interest, potentially confounding the results. Therefore, it is desirable to develop less invasive methods of light generation in the brain for *in vivo* optogenetics. One potential method to stimulate *in vivo* optogenetics would use X-ray activation of radioluminescent materials. X-rays are ideally suited for use in optogenetics because the beam can maintain focus through the tissue with little scattering. However, high levels of X-irradiation have been shown to cause cognitive dysfunction and neuronal death. Little is known about how lower levels of X-irradiation affects synaptic transmission. In this study, extracellular and whole cell electrophysiology methods are used to investigate the acute effects of X-rays on neuronal health and overall circuit function at various doses, up to 5 Gy. In preliminary experiments from cell culture, low levels of X-ray exposure alone did not depolarize neurons or stimulate synaptic transmission. Furthermore, in acute hippocampal slices, low levels of X-rays had no effect on basal synaptic transmission. However, the highest dose used did cause a modest reduction of basal synaptic transmission. Next, we tested for effects of X-irradiation on long-term potentiation, a more robust measurement of synaptic health and integrity. Long-term potentiation could still be induced at all levels of X-ray tested. Together, these results indicate that neuronal function and synaptic plasticity are intact during low levels of X-ray exposure. Because X-rays have been shown to cause low level activation of rhodopsin in the retina, we will verify that a low level of X-ray exposure does not itself cause activation of an opsin such as channelrhodopsin-2. In addition, we will test the ability of visible light emitted from radioluminescent materials to activate the opsin. Therefore, these experiments will provide proof of principle that the use of X-ray to activate radioluminescent materials would potentially be a viable tool for noninvasive delivery of light for *in vivo* optogenetics.

Disclosures: **A.F. Bartley:** None. **J.A. Barnes:** None. **D.N. French:** None. **T.R. Totsch:** None. **G.M. Gray:** None. **L.L. McMahon:** None. **L.E. Dobrunz:** None.

Poster

253. Optogenetics I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 253.12/BB70

Topic: I.08. Methods to Modulate Neural Activity

Support: ERC Grant #677683
ERC Grant #692943
NIH Grant U01NS094190

Title: Tapered fiber optrode for simultaneous ChR2 activation and electrical recording over small brain volumes

Authors: ***B. SPAGNOLO**[1], L. SILEO[1], R. PEIXOTO[2], M. PISANELLO[1], F. PISANO[1], E. MAGLIE[1,3], B. L. SABATINI[4], F. PISANELLO[1], M. DE VITTORIO[1,3];
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Abstract: Optogenetic control of neural activity is a widely used approach to study brain circuitry and dissect neural pathways in order to understand how different brain areas communicate and cooperate in determining animal behaviour. Molecular tools to genetically target specific neural subpolpulations and opto-electronic devices can be used in different combinations to address small or wide brain volumes for optical control and electrically monitor brain activity [1]. By virtue of their low invasiveness and precisely tunable light delivery properties, tapered optical fibers (TFs) have been demonstrated as an extremely versatile tool for triggering brain activity, but they still require a second device for electrical readout of cell activity so far [2-6].
We here combine different fabrication technologies (Focused Ion Beam, Ion Induced Beam Deposition) to pattern the non-planar surface of metalized TFs to realize a fiber-optrode featuring a platinum electrode integrated in close proximity to an optical active site (window). Both the electrode and the window size can be easily customized in terms of size and shape, while their mutual position has been kept constant in this study to reduce direct electrode illumination. Devices have been characterized *in vitro* for impedance measurements and light emission properties, and also tested for *in vivo* optogenetic stimulation.
In vivo experiments demostrate that the fiber-optrode can be successfully used for simultaneous light delivery and electrical readout of brain activity in awake head-restrained mice with no photoelectric effect in the high-frequency spikes channel. Results also show that neural response,

in terms of number of spikes, likely increases as the window size and related area of optogenetic activation increase. This approach allows for high reproducibility and straightforward tailoring of optical windows and electrode sizes, thus greatly increasing device versatility upon various experimental needs and brain areas of interest.

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[6] F. Pisano et al., Microelectronic Engineering 195, 41-49 (2018)

Disclosures: **B. Spagnolo:** None. **L. Sileo:** None. **R. Peixoto:** None. **M. Pisanello:** None. **F. Pisano:** None. **E. Maglie:** None. **B.L. Sabatini:** None. **F. Pisanello:** None. **M. De Vittorio:** None.

Poster

253. Optogenetics I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 253.13/BB71

Topic: I.08. Methods to Modulate Neural Activity

Support: University of Arizona start up fund

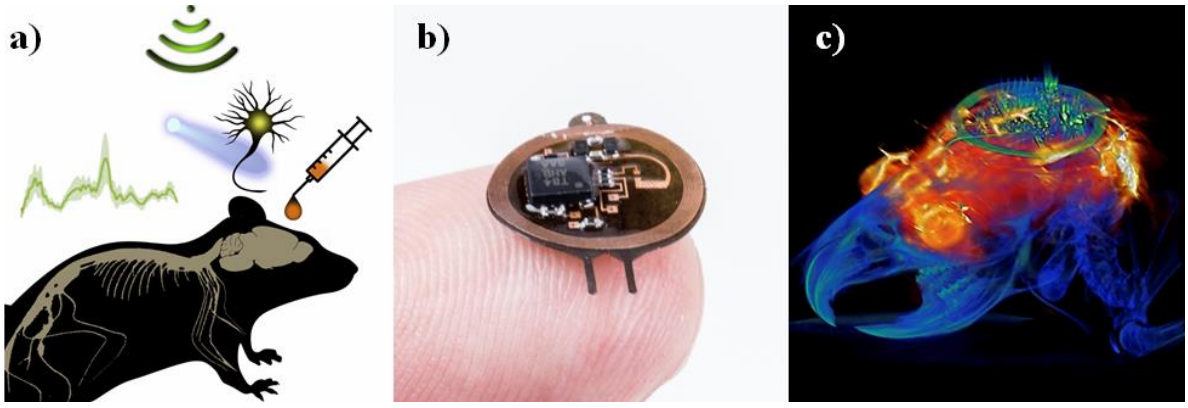
Title: Wireless, subdermally implantable neuromodulation tools for chronic recording and stimulation in freely moving subjects

Authors: A. K. BURTON, J. AUSRA, T. STUART, ***P. GUTRUF**;
Univ. of Arizona, Tucson, AZ

Abstract: Recently emerging classes of battery free, ultrasmall, fully implantable devices for optogenetic neuromodulation eliminate physical tethers associated with bulky head-stages and batteries in alternative wireless technologies and conventional setups by leveraging cellular scale light emitting diodes on flexible injectable filaments as light sources. These highly miniaturized systems enable untethered, operation for behavioral studies that eliminate motion constraints and enable new experimental paradigms in a range of complex 3D environments and contexts that cannot be explored with conventional technologies. Here we show concepts that enable controlled device operation, independent of position and angle relative to the experimental arena, with advanced wireless power harvesting capabilities and full user-programmability over multiple devices that allow for indefinite experimentation in paradigms featuring multiple subjects (Figure 1 a and b). This level of functionality is demonstrated in integrated platforms that are compatible with noninvasive imaging technologies such as computed tomography (CT)

and magnetic resonance imaging (MRI) (Figure 1 c). We extend this concept to devices with capabilities in multimodal optogenetic stimulation and genetically targeted calcium recording of the brain and the peripherals resulting in a broad suite of modulation and recording tools for the nervous system and major organs such as the heart.

Figure 1. Wireless battery free subdermally implantable neuromodulation and recording tools A. Schematic illustration of multimodal recording and stimulation B. Photographic image of a digitally controlled multimodal optogenetic stimulation tool C. 3D rendering of superposed MRI and CT image analysis of a subject implanted with the tool shown in B.



Disclosures: **A.K. Burton:** None. **J. Ausra:** None. **T. Stuart:** None. **P. Gutruf:** None.

Poster

253. Optogenetics I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 253.14/BB72

Topic: I.08. Methods to Modulate Neural Activity

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National Key R&D Program of China.2017YFC0840100 and 2017YFC0840106
Technology Innovation Program of Shanghai Science and Technology Committee.18411950100

Title: A shape-memory and spiral light-emitting device for precise multisite stimulation of nerve bundles

Authors: *H. ZHENG[1], Z. ZHANG[2], S. JIANG[1], B. YAN[1], X. SHI[2], Y. XIE[1], X. HUANG[1], Z. YU[3], H. LIU[1], S. WENG[1], A. V. NURMIKKO[4], Y. ZHANG[2], H. PENG[2], W. XU[1], J. ZHANG[1]; [1]State Key Lab. of Med. Neurobiology, Inst. of Brain Science, MOE Frontier Ctr. for Brain Science, Dept. of Hand Surgery, Natl. Clin. Res. Ctr. for Aging and Medicine, Huashan Hospital; Fudan Univ., ShangHai, China; [2]State Key Lab. of Mol. Engin. of Polymers, Dept. of Macromolecular Science, and Lab. of Advanced Materials; Fudan Univ., ShangHai, China; [3]Sch. of Engineering, Brown Univ., Providence, RI; [4]Sch. of Engin., Brown Univ., Providence, RI

Abstract: We previously demonstrated that for long-term spastic limb paralysis, transferring the seventh cervical nerve (C7) from the nonparalyzed side to the paralyzed side results in increase of 17.7 in Fugl-Meyer score. One strategy for further improvement in voluntary arm movement is selective activation of five target muscles innervated by C7 during recovery process. In this study, we develop an implantable multisite optogenetic stimulation device (MOSD) based on shape-memory polymer. Two-site stimulation of sciatic nerve bundles by MOSD induces precise extension or flexion movements of the ankle joint, while eight-site stimulation of C7 nerve bundles induce selective limb movement. Long-term implant of MOSD to mice with severed and anastomosed C7 nerve is proven to be both safe and effective. Our work opens up the possibility for multisite nerve bundle stimulation to induce highly-selective activations of limb muscles, which could inspire further applications in neurosurgery and neuroscience research.

Disclosures: H. Zheng: None. **Z. Zhang:** None. **S. Jiang:** None. **B. Yan:** None. **X. Shi:** None. **Y. Xie:** None. **X. Huang:** None. **Z. Yu:** None. **H. Liu:** None. **S. Weng:** None. **A.V. Nurmikko:** None. **Y. Zhang:** None. **H. Peng:** None. **W. Xu:** None. **J. Zhang:** None.

Poster

253. Optogenetics I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 253.15/BB73

Topic: I.08. Methods to Modulate Neural Activity

Support: Wellcome Trust and Royal Society Fellowship 109372/Z/15/Z

Title: Ultrafast and spatially precise evoked and quantified protective pain behaviour

Authors: A. SCHORSCHER-PETCU, ***L. E. BROWNE**;
Univ. Col. London, London, United Kingdom

Abstract: Nociception serves a vital function by alerting us to the presence of harmful stimuli and triggering fast protective behaviors and pain. The primary afferents that innervate skin and drive these responses are heterogenous, encoding a wide range of sensory modalities. Added to this complexity, stimuli cannot be applied with high enough spatial (sub-millimeter) and

temporal (sub-second) precision to match the timescale on which these behaviors occur. To address these challenges, here we have developed skInsight, an ultrafast and spatially precise quantitative behavioral platform that provides remote optogenetic activation of primary afferent peripheral terminals in freely-behaving mice. The system can activate genetically-targeted cutaneous terminals in a 150 um diameter spot, with a time-locked laser pulse as short as 100 us. Concomitant high-speed video recordings at 1000 frames per second are subsequently analyzed with custom software, allowing for objective detection of nocifensive behavior at the millisecond timescale. This automated analysis enables us to efficiently dissect and rapidly quantify distinct types of protective behaviors otherwise not discernible by simple observation: from local responses that occur below the withdrawal threshold or vary in timing, up to rapid whole-body repositioning behaviors. We validated the system in mice expressing ChR2 in a broad class of primary afferent nociceptors and addressed how varying a single optical stimulation affects the probability, magnitude and diversity of protective behavior. Next, we investigated how the shape and density of a patterned optical stimulus affects withdrawal behaviors. These experiments enabled us to define the minimal input size required to trigger protective behaviors and identify that repositioning behaviors are highly predictive of stimulus intensity. This behavioral system allows us to probe the sensory-motor input-output relationship of genetically-defined cutaneous afferent subpopulations with unprecedented spatial and temporal precision. Combined with other neurophysiological techniques, skInsight can be employed to understand how neural circuits are recruited by both noxious and innocuous sensory inputs and drive a specific adaptive behavioral response.

Disclosures: L.E. Browne: None. **A. Schorscher-Petcu:** None.

Poster

253. Optogenetics I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 253.16/BB74

Topic: I.08. Methods to Modulate Neural Activity

Support: NSF, EPSCoR, Grant 1632881
Alabama EPSCoR GRSP

Title: Noninvasive optogenetics using MRI guided focused ultrasound delivery of radioluminescent nanoparticles

Authors: *M. C. RICH[1], E. ZHANG[2], M. K. BURDETTE[2], A. DICKEY[2], K. E. CANNON[1], S. H. FOULGER[2], M. S. BOLDING[1];
[1]Dept. of Radiology, Univ. of Alabama Birmingham, Birmingham, AL; [2]Dept. of Materials Sci. and Engin., Clemson Univ., Anderson, SC

Abstract: The ability to noninvasively activate, silence, and provide receptor subtype specific neuromodulation with high temporal resolution and spatial specificity would greatly advance our ability to study brain circuits in vivo. Optogenetics, the genetic incorporation of light sensitive proteins such as Channelrhodopsin-2 (ChR2) into target mammalian neurons, has met nearly all of these criteria. However, the essential components of the optogenetic system require invasive procedures with very few noninvasive alternatives. In order to achieve location specific delivery of viral vectors for genetic expression of opsin proteins, invasive surgical infusions are required. Furthermore, the implantation of light emitting fibers deep within brain structures is both technically demanding and causes additional tissue destruction and scarring in target brain regions. Glial scarring at the light source can decrease the effectiveness of light intensities leading to variability in channel activation. In addition, the light intensities required to activate neurons with fiber optic delivery can result in local heating of the brain tissue, potentially leading to thermal ablation and/or unwanted physiological effects. To overcome these limitations, we are replacing fiber optic implants with light-emitting radioluminescent particles (RLPs) that can be activated noninvasively with X-ray exposure. Here, we report noninvasive delivery of RLPs to target brain regions with MRI-guided focused ultrasound (FUS) blood brain barrier opening (BBBO). In addition, FUS BBBO can be used to deliver viral vectors for light sensitive channel expression. Combined, these components can provide a completely noninvasive optogenetic system.

Disclosures: M.C. Rich: None. **E. Zhang:** None. **M.K. Burdette:** None. **A. Dickey:** None. **K.E. Cannon:** None. **S.H. Foulger:** None. **M.S. Bolding:** None.

Poster

253. Optogenetics I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 253.17/BB75

Topic: I.08. Methods to Modulate Neural Activity

Support: DARPA W911NF-17-C-0059

Title: Neural recording and stimulation technologies for *in vivo* electrophysiology

Authors: *J. C. MORIZIO[1], A. DESHMUKH[2], V. GO[1];
[2]Res. and Develop., [1]Triangle Biosystems Intl., Durham, NC

Abstract: Over the last decade wireless technology advancements for neural recording and stimulation have evolved whereby they can now be designed for implantable or head mounted *in-vivo* electrophysiology applications on freely moving small rodents to non-human primates. System level concepts for implantable or head mounted telemetric recording up to 256 simultaneous channels and bipolar constant current electrical stimulation on 16 channels will be

described. Key design challenges and tradeoffs of these wireless technologies will be explained. We will present integrated systems solutions for head mounted and implantable technologies to acquire EEG, EMG, ECG and single units or spikes signals from brain, central nerve or peripheral nerves. Sub-system components and accessories will also be described that include electrodes or neural interfaces, low noise integrated CMOS electronics, RF circuitry, inductive powering and DAC analysis software used for neural recording. The same will follow for constant current electrical stimulation and optogenetic stimulation and combo stimulation/recording headstage implantable technologies.

Disclosures: J.C. Morizio: None. **A. Deshmukh:** None. **V. Go:** None.

Poster

253. Optogenetics I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 253.18/BB76

Topic: I.08. Methods to Modulate Neural Activity

Support: PRESTO JPMJPR178C

Title: Adaptive brain machine interface with somatosensory feedback potential component technology developments

Authors: *F. YOSHIDA[1], M. HIRATA[2];
[1]Saga Univ., Saga, Japan; [2]Osaka Univ. Clin. Neuroeng, Suita, Japan

Abstract: Position or touch sense is important for clinical applications of the BMI because ideal prosthetic limbs should be perceived as natural extensions of the users' bodies. We have started to design a cortical modulator using optogenetics-a new method for the manipulation of neurons to dial in potential sensory input in a bi-directional manner. Optogenetics is based on genetically modified ion channels that respond directly to light. These light-gated ion channels, such as Channelrhodopsin-2 (ChR2), allow precise, millisecond control of specific neurons. This technique reduces most of the key problems associated with electrical brain stimulation: there is no associated electrical artifact to interfere with the electrophysiological recordings, nor any tissue damage from the current injection. It also allows for precise control of the spatial pattern of stimulation. A prototype optogenetic implant is presented, that will simultaneously record the activity of cortical neuronal activities and bring complex modulation patterns through optogenetic stimulation of cortical sensory areas. Our newly-invented optogenetic devices consist of both ECoG and LED for optical stimulation. Here we report data from initial bench testing and implantation for the ECoG in both the rat and non-human primate. We have show that the ECoG is effective as a chronic implant in rats, providing high fidelity neural recordings for up to 8 weeks. The initial results suggest that the new ECoG array can be successfully

translated from rodents to accommodate the technological challenges associated with successfully interfacing with the non-human primate brain.

Disclosures: F. Yoshida: None. **M. Hirata:** None.

Poster

253. Optogenetics I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 253.19/BB77

Topic: I.08. Methods to Modulate Neural Activity

Support: MSTP T32 Grant T32GM007308

Title: The role of sparse neural subsets in driving choice behavior: An optical microstimulation study

Authors: *R. PANCHOLI, S. P. PERON;
Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Perturbation experiments employing microstimulation have been instrumental in drawing causal relationships between specific areas of cortex and perception. These studies, along with those demonstrating the sparsity of stimulus representations in primary sensory areas, suggest that mammalian perception is underpinned by sparse subsets of neurons within a defined sub-region of the brain. Despite this, it remains unclear how small groups of cells contribute to choice behavior and to perception. This uncertainty is largely the result of limitations of current microstimulation methods, including stimulus artifacts, unreliable inter-session reproducibility, and the lack of spatial and genetic targeting, which make concurrent recording challenging and interpretation difficult. To address this issue, we have developed an all-optical approach combining genetically-targeted one-photon photostimulation and cellular-resolution two-photon calcium imaging in awake, behaving transgenic mice expressing GCaMP6s. By delivering soma-localized opsin to layer 2/3 of a single barrel in primary vibrissal somatosensory cortex and affixing a light emitting diode to the surface of a cranial window, we create a source of light that can drive the opsin stably and reproducibly over the course of weeks to months. This optical microstimulation approach overcomes prior methodological limitations and allows for full characterization of evoked activity in the opsin-expressing tissue as well as in downstream areas. It also facilitates subsequent cellular-resolution optical perturbations. In particular, we use this paradigm to ask whether a sparse subset of neurons in a primary sensory area can drive choice behavior. Preliminary experiments show that we can stimulate and record from infected neurons in animals performing a two-lickport, yes-no, optical microstimulation task and perform single-cell gain- and loss-of-function optical perturbations. Further experiments will explore the behavioral and network-level effects of these optical perturbations and draw comparisons

between the photostimulus-evoked activity and naturalistic activity evoked in a vibrissal, object-localization task.

Disclosures: R. Pancholi: None. **S.P. Peron:** None.

Poster

253. Optogenetics I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 253.20/BB78

Topic: I.08. Methods to Modulate Neural Activity

Support: HHMI
NIH 1UF1NS107574-01
ANR-05-RNTL-0023
Fondation Bettencourt Schueller
Axa Research Fund

Title: High throughput *in vivo* synaptic connectivity mapping with volumetric two-photon optogenetics and Ca^{2+} imaging in zebrafish

Authors: D. TANESE[1], A. ABDELFATTAH[2], L. LAVIS[2], E. SCHREITER[2], V. EMILIANI[1], ***M. KOYAMA**[2];
[1]Inst. de la Vision, Paris, France; [2]HHMI Janelia Res. Campus, Ashburn, VA

Abstract: Synapses conduct signals between neurons and allow neurons to form circuits that ultimately control animal behavior. Their strength changes dynamically over a range of timescale from milliseconds to days. Such dynamical change has been suggested to underlie various computations and plasticity in the brain. Although paired intracellular recording has been routinely used to measure such dynamics, its use has been restricted to small-scale analyses in acute experiments due to its low throughput and invasiveness. Here we developed a system for volumetric two-photon optogenetics and Ca^{2+} imaging in zebrafish and demonstrated its use for synaptic connectivity mapping. We used holographic stimulation to activate neurons expressing soma-localized CoChR at 950 nm and confirmed their activation with two-photon volumetric imaging of jRGECO1b at 1140 nm with remote-focusing. This combination of opsin and Ca^{2+} indicator minimized the imaging-induced opsin activation down to a few millivolts at most. Simultaneous volumetric Ca^{2+} imaging also revealed occasional off-target activation of neurons, indicating the importance of confirming the activated cells when 2p optogenetics is used to map synaptic connectivity at a single-cell level. In combination with whole-cell recordings from spinal motoneurons, we mapped the connectivity of spinal V2a neurons to motoneurons and revealed a class of V2a neurons with long-range connections that span across more than 10 segments. We are in the process of combining this system with voltage imaging with a

chemigenetic voltage indicator, Voltron, to detect synaptic inputs optically. Preliminary results showed that it is possible to detect synaptic potentials as small as a few millivolts, opening up the possibility to optically monitor the dynamics of many synaptic connections for an extended period of time in vivo.

Disclosures: D. Tanese: None. **A. Abdelfattah:** None. **L. Lavis:** None. **E. Schreiter:** None. **V. Emiliani:** None. **M. Koyama:** None.

Poster

254. Novel Approaches in Neuromodulation I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 254.01/BB79

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH/NIDA Intramural Research Program
NIH Grant ZIA000069

Title: Advancing translational chemogenetics with positron emission tomography

Authors: *M. A. BOEHM[1,2], H. JEDEMA[1], J. BONAVENTURA[1], J. GOMEZ[1], E. STEIN[1], M. MICHAELIDES[1], C. BRADBERRY[1];
[1]Natl. Inst. on Drug Abuse, Baltimore, MD; [2]Brown Univ., Providence, RI

Abstract: The ability to systematically manipulate brain activity in vivo is essential for understanding its contribution in brain function, behavior and disease. Chemogenetic technologies offer a minimally invasive approach for modulating neural activity. Verifying the expression, location and function of chemogenetic receptors following transduction is a necessary practice, and the ability to do this in vivo is critical for chemogenetic applications in nonhuman primates and humans. Positron emission tomography (PET) is a translational molecular imaging modality that is uniquely poised to enable in vivo confirmation of chemogenetic tools. As with any imaging modality, PET imaging methods need to be tailored for different species and questions of interest. Here we lay the groundwork for assessing the use of PET for in vivo confirmation of chemogenetic applications in squirrel monkeys (*Saimiri sciureus*). First we performed PET scans following intravenous injections of [^{18}F]-fluorodeoxyglucose (FDG), a metabolic indicator of brain activity. To test the reproducibility of FDG brain uptake after a bolus injection, monkeys underwent two baseline scans separated by a week or more. Within-subject comparisons indicate acceptable reproducibility (8.4% ± 3.7% difference in mean SUVs, n=2) and rapid uptake of FDG in the brain, with over 95% occurring by 5 min post injection. To assess the feasibility of measuring continuous changes in brain activity, we performed dynamic PET scans in monkeys (n=3) receiving a continuous infusion of FDG over a 90 min period. We observed a stable linear increase in FDG brain signal for all

scans, indicating this method is viable for monitoring changes in brain activity over time. Next we began testing the use of recently developed ligands for Designer Receptors Exclusively Activated by Designer Drugs (DREADDs). We examined effects of the potent DREADD agonist JHU37160 (0.1 mg/kg) on brain activity in DREADD-naïve monkeys. Initial results suggest JHU37160 does not produce off-target effects on brain activity, with no significant changes compared to baseline scans (n=4). We also examined the use of [^{18}F]-JHU37107, a ligand designed for visualizing DREADD receptor expression with PET. To assess the time-course and baseline distribution of [^{18}F]-JHU37107 in the brain, we scanned a DREADD-naïve monkey for 120 min following a bolus injection of the compound. [^{18}F]-JHU37107 signal in the brain peaked at 20 min post injection and remained detectable after 2 hours. Overall these preliminary findings demonstrate the feasibility of PET-assisted in vivo confirmation for chemogenetic applications in squirrel monkeys. (Supported by NIH-NIDA IRP)

Disclosures: M.A. Boehm: None. **H. Jedema:** None. **J. Bonaventura:** None. **J. Gomez:** None. **E. Stein:** None. **M. Michaelides:** None. **C. Bradberry:** None.

Poster

254. Novel Approaches in Neuromodulation I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 254.02/BB80

Topic: I.08. Methods to Modulate Neural Activity

Support: Canadian Institutes of Health Research
Fonds de recherche du Québec - Santé

Title: Signalings signature of DREADDs: Are they completely biased receptors?

Authors: *É. BESSERER-OFFROY[1], J.-M. LONGPRÉ[2,3], T. E. HÉBERT[1], P. SARRET[2,3]; [1]Dept. of Pharmacol. and Therapeut., McGill Univ., Montreal, QC, Canada; [2]Pharmacol. and Physiol., Univ. de Sherbrooke, Sherbrooke, QC, Canada; [3]Inst. de Pharmacologie de Sherbrooke, Sherbrooke, QC, Canada

Abstract: Designer **R**eceptors **E**xclusively **A**ctivated by **D**esigner **D**rugs (DREADDs) are seven transmembrane receptors (7TMR) engineered to be activated specifically by Clozapine-N-Oxide (CNO), a drug which has no known biological activity by itself. These DREADDs were originally derived from members of the muscarinic receptor family to only bind CNO, and no longer respond to their biological ligand, acetylcholine. DREADDs are widely used by neuroscientists to hijack cell signaling and to decipher *in vivo* physiological relevance of these signaling pathways. To date, no study has examined their molecular signaling signatures to confirm that these receptors show absolute functional selectivity for particular signaling pathway, acting as truly biased DREADDs. In this study, we investigated the signaling

signatures of the three DREADDs, hM3D, rM3D and hM4D characterized respectively as G_q, G_s and G_i activators. We were also interested to characterize the recently reported β-arrestin DREADD, hM3D-R165L. We used BRET-based biosensors to monitor G protein activation (G_q, $G_{i1,2,3}$, $G_{oA,B}$, and $G_{12,13}$) and β-arrestin 1/2 recruitment in HEK 293 cells, a heterologous line widely used because of ease of transfection. As DREADDs are often used in neurosciences, we also investigated DREADD signaling in DRG/F11 cells, a hybridoma of F11 mouse neuroblastoma and embryonic rat dorsal root ganglion neurons, as a cell line representative of primary sensory neurons. Our results, in HEK 293 cells, showed that hM3D (G_q) is the only DREADD able to activate the G_q biosensor. This receptor was also able to activate, to a lesser extent, G_{13} and G_{i1}. The G_s DREADD (rM3D), was able to trigger cAMP formation after stimulation with CNO, but also activated the G_{13} biosensor with an efficacy similar to hM3D. The G_i DREADD, hM4D, induced the activation of $G_{i1,2,3}$ and $G_{oA,B}$. Interestingly, in HEK 293 cells, this G_i DREADD also promoted cAMP production with the same efficacy but lower potency than its G_s counterpart. Furthermore, hM4D activated the G_{13} biosensor in a similar manner as the two other DREADDs. Finally, all three DREADDs were found to promote recruitment of β-arrestins 1 and 2 to the plasma membrane after treatment with CNO. However, this recruitment occurred at high concentration of CNO. In conclusion, DREADDs represent important chemogenetic tools for neuroscientists. Nevertheless, we should exercise caution when defining them with a signaling pathway as these DREADDs may not be purely biased receptors as initially thought.

Disclosures: É. Besserer-Offroy: None. **J. Longpré:** None. **T.E. Hébert:** None. **P. Sarret:** None.

Poster

254. Novel Approaches in Neuromodulation I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 254.03/BB81

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH/NIDA DA045284-01A1
Whitehall Foundation Research Grant

Title: Decoding the functional role of the ventral tegmental area-olfactory tubercle dopamine circuit via integration of electrochemical and chemogenetic techniques

Authors: *R. BHIMANI[1], C. E. BASS[2], J. PARK[1];
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Abstract: The olfactory tubercle (OT), a limbic structure located at the ventral most portion of the ventral striatum, receives dense dopamine (DA) innervation from the ventral tegmental area

(VTA) and plays a pivotal role in the reinforcing effects of drugs of abuse. However, due to its anatomical location and proximity to neighboring DA rich brain structures (e.g. nucleus accumbens, caudate putamen), functional characterization of OT-DA has been limited. In order to overcome such challenges, recent genetic techniques such as Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), have facilitated the understanding of the functional roles of complex brain circuits. DREADDs are synthetic G-protein coupled receptors that can be delivered to neurons via gene transfer and allow for the excitation or inhibition of neuronal activity through pharmacologically inert ligands such as Clozapine-N-Oxide (CNO). In this study, we used a viral targeting system to restrict DREADD expression to VTA-DA neurons and employed *in vivo* fast-scan cyclic voltammetry (FSCV) to determine how CNO modulates DA transmission and associated behavioral outputs in awake behaving rats. We further compared these findings with a retrograde intersectional viral approach to selectively modulate VTA-DA projections neurons that innervate the OT. Through immunohistochemical and electrochemical evidence, we demonstrate how chemogenetic modulation of VTA-DA neurons impacts sub-second changes in DA transmission in the OT and compared the effects of global and discrete sub-populations of VTA-DA neuron modulation. These findings will provide a novel understanding of the VTA-DA-OT circuit and how it is linked to various brain functions as well as psychiatric and neurodegenerative diseases.

Disclosures: **R. Bhimani:** None. **C.E. Bass:** None. **J. Park:** None.

Poster

254. Novel Approaches in Neuromodulation I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 254.04/BB82

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: 80NSSC17K0060 (NASA; AJE)
CHOP Department of Anesthesiology and Critical Care Development Funds (AJE)
PENN McCabe award (SY)

Title: The effect of chronic systemic administration of the DREADD agonists clozapine-N-oxide (CNO) and Compound 21 (C21) on mouse behavior

Authors: ***F. H. TRAN**[1], K. J. ANH[1], S. YUN[1,2], A. J. EISCH[1,2];
[1]Dept. of Anesthesiol. and Critical Care Med., Children's Hosp. of Philadelphia, Philadelphia, PA; [2]Univ. of Pennsylvania, Philadelphia, PA

Abstract: The use of chemogenetics to remotely control the activity of neuronal populations is widely-used to study rodent brain circuit function. Designer Receptors Exclusively Activated by

Designer Drugs (DREADD) is a subclass of chemogenetics where engineered muscarinic receptors permit control of G-protein signaling via administration of synthetic ligands such as clozapine-N-oxide (CNO). Although CNO is the most commonly-used ligand for DREADD receptors, CNO is back-metabolized to clozapine which itself activates numerous endogenous receptors. To eliminate potential off-target effects of CNO, a new DREADD agonist - Compound 21 (C21) - has been proposed as a possible alternative as it lacks active metabolites. Despite published work comparing acute exposure of C21 vs. CNO on mouse behavior, little is known about the behavioral effects of chronic administration of either compounds. Here we tested whether chronic administration of these two DREADD agonists change key behavioral indices in mice relative to a vehicle injection group. Vehicle (Veh, 0.5% DMSO/Sal), CNO (0.2 mg/ml, 1mg/kg), or C21 (also 0.2 mg/ml, 1mg/kg) were injected intraperitoneally in CamKIIalpha-icre male mice daily for 20 weeks. For locomotion, all three groups showed similar infrared beam breaks during a 30-minute session. Similarly, there were no effects on anxiety-like behavior; elevated plus maze testing showed all mice spent similar time in the closed arms and had similar latency to enter closed arms, and open field testing showed all mice spent similar time in the peripheral zone. One behavioral difference among the groups was observed: in the open field, the latency to enter center was on average ~8 s longer for C21 vs. CNO mice. Taken together, these data show chronic C21 and CNO produce quite similar results to Veh on locomotor activity and anxiety-like behaviors. We are currently exploring the temporal action of chronic C21, CNO, and Veh on the above behaviors, as well as measuring social interaction, stereotypy, and indices of despair. Together, these analyses will provide important guidance for future chemogenetic experiments where chronic CNO or C21 is used, and may even help provide alternative interpretations for previously published work.

Disclosures: **F.H. Tran:** None. **K.J. Anh:** None. **S. Yun:** None. **A.J. Eisch:** None.

Poster

254. Novel Approaches in Neuromodulation I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 254.05/BB83

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH/NINDS Grant R01NS072171

Title: Peripheral nerve injury decreases spontaneous and evoked neural activity in the somatosensory cortex

Authors: *V. KRISHNAN[1,2], G. PELLED[1,2,3];
[1]Biomed. Engin., [2]Inst. for Quantitative Hlth. Sci. and Engin., [3]Dept. of Radiology, Michigan State Univ., East Lansing, MI

Abstract: 20 million Americans suffer from peripheral nerve injury that leads to significant changes in cortical and subcortical neuronal activity. There is also a paucity of evidence to predict recovery, since the exact mechanisms of brain plasticity changes following injury is still at its infancy. We have prior evidence from our lab that peripheral nerve injury results in an increase in activity of inhibitory interneurons in layer 5 of the somatosensory cortex in the affected (deprived) cortex(Han et al., *Neurorehab and Neur Repair* 2013; Pelled et al., *PNAS* 2011; Li et al., *PNAS* 2011). This mechanism is thought to be mediated through the transcallosal pathway. We are utilizing electrophysiology methods (patch clamping) in order to detect neuronal changes in the cortex occurring in the single neuron and network level. We performed complete forepaw denervation in 4-week-old rats. Intracellular recordings were performed two weeks after denervation. Whole-cell patch clamp recordings from both morphologically identified excitatory (pyramidal) and inhibitory neurons in layer 5 in the affected cortex were collected with and without stimulation of the transcallosal projections. Bipolar tungsten electrode serves for delivering high frequency stimulation were placed in corpus callosum. Recording patch electrode were placed contralateral to injured limb to monitor changes after stimulation. The mean number of EPSCs before transcallosal stimulation was 3.33 ± 1.85 in denervated rats (n=3) compared to 6 ± 1.29 in control rats (n=7). This shows a reduced spontaneous neuronal activity in rats with peripheral nerve injury compared to control rats.
We then applied high-frequency stimulation of 100 Hz to the transcallosal fibers. Stimulation increased the number of EPSCs by 25.4 ± 12.99 % in denervated rats and 168.1 ± 43.8 % in control rats. In addition, long term potentiation (LTP) was determined by measuring changes in amplitude of EPSCs before and after stimulation. Preliminary results demonstrate that the pre-stimulus EPSC amplitude in control rats was 16.84 ± 4.31 pA, and the stimulation led to significant increases in the amplitude of 69.1 ± 17.39 pA (p=0.02). However, in denervated rats, stimulation led to 11.02 ± 15.27 % decrease in post-stimulus EPSC amplitude. Together, these results suggest that the injury decreases the probability of inducing LTP in excitatory cortical neurons both by suppressing the amplitude and the number of EPSCs events. We plan to record from multiple neuronal population and study their role in post-injury plasticity.

Disclosures: **V. Krishnan:** None. **G. Pelled:** None.

Poster

254. Novel Approaches in Neuromodulation I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 254.06/BB84

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH NINDS Grant R01NS098231

Title: Non-invasive, magnetogenetics stimulation of rat primary visual cortex

Authors: *A. FARNUM[1,2], G. PELLED[1,2,3];
[1]Biomed. Engin., [2]Inst. for Quantitative Hlth. Sci. and Engin., [3]Dept. of Radiology, Michigan State Univ., East Lansing, MI

Abstract: There are an estimated 253 million blind or severely visually impaired people in the world today. Electrode-based neuroprosthetics have been developed to stimulate specific neural regions for a diverse range of diseases, including vision loss. However, insertion of electrodes often induces short and long term changes that can negatively impact cortical function. The goal of this research is to develop a next-generation non-invasive and cell-specific visual neuromodulation. We are developing a novel technology based on the electromagnetic-perceptive gene (EPG), which we recently isolated from the glass catfish, *Kryptopterus bicirrhis* (Krishnan et al, *Sci Reports,* 2018). This gene has been shown to respond to electromagnetic fields. We engineered a subpopulation of neurons in the adult Long Evans rat primary visual cortex (V1) to express the EPG via unilateral stereotaxic virus injections (AAV-CamKII-EPG-IRES-GFP)(n=4). Two weeks after the injection we anesthetized the rats with urethane and used in-vivo electrophysiology to record neuronal action potentials in V1 using multi-electrode arrays. Multi-unit activity (MUA) were sampled at 10.5 k Hz and band-pass filtered between 300-10 k Hz. Discriminated signals were collected from a CED interface and Spike2 software. Spike sorting was performed on Spike2. Post-stimulus time histogram analysis was performed to define stimulus-evoked spiking activity. MUA that was greater than 2.5 times the standard deviation in one 50-ms bin during the first 1 sec following the onset of the stimulation were considered stimulus-evoked responses. Green light stimulus that was delivered for 1 sec to one eye through an LED, evoked significant increase of MUA responses (115%) in the contralateral V1 with a 75±25 ms delay. We found that electromagnetic stimulation of 6 mT for 1 sec also evoked a significant increase in neuronal responses (227±58%) in the EPG-expressing V1 with a 275±75 ms delay. The V1 without EPG of the same individuals did not exhibit any change in neuronal activity in response to electromagnetic stimulation. Our results demonstrate that the EPG-based neuromodulation evoked visual responses in anesthetized rats and has the potential to be an effective approach to remote-control visual cortex function. We are now working on optimizing the technology and testing it in awake-behaving animals.

Disclosures: A. Farnum: None. **G. Pelled:** None.

Poster

254. Novel Approaches in Neuromodulation I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 254.07/BB85

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant R01NS098231

NIH Grant R01NS104306
NIH Grant R01NS098231

Title: Putative role of the cannabinoid receptor and G protein in electromagnetic perceptive gene (EPG) mediated calcium activation evoked by magnetic field in mammalian cells

Authors: *S. XU[1,2], Z. M. KRANZ[3], X. ZHANG[1,2], S. MITRA[1,2], G. PELLED[1,2,4], A. A. GILAD[1,2,4];
[1]Biomed. Engin., [2]Inst. of Quantitative Hlth. Sci. and Engin., [3]Col. of Osteo. Med., [4]Dept. of Radiology, Michigan State Univ., East Lansing, MI

Abstract: Recently, we have identified and cloned the Electromagnetic Perceptive Gene (EPG) from the *Kryptopterus bicirrhis* (glass catfish). We have expressed the EPG in mammalian cells and demonstrated that in the presence of magnetic field there are increases of intracellular calcium (Krishnan et al., Sci Reports, 2018). This feature makes EPG a potential new technology for remote control of cellular activity. However, the mechanism by which the magnetic field activates the EPG remains to be identified. To find what proteins EPG interact with in mammalian cells, a glutathione S-transferase (GST) tag was introduced to the N-terminal of EPG which improves the protein expression and solubility in E. coli. The affinity-purification was followed by mass spectrometry (AP-MS) with the enrichment of GST tag. Pulldown was performed using extract both from rat brain and HEK293 cell. Additionally, formaldehyde crosslink immunoprecipitation with and without magnetic field was used to identify the binding partners of the EPG. Using bioinformatics, three candidates out of 300 AP-MS reads were tested: G Protein Subunit Alpha I3 (Gαi3) and O1 (Gαo), and the cannabinoid receptor 1 (CB1) which are expressed in the cerebral cortex. Moreover, the affinity between EPG and its partners appeared to be enhanced in the presence of magnetic field: the immunoprecipitation showed the CB1 pulldown by EPG under a magnetic field of 100 mT increased by 190 ±10 %. We then investigated this signal transduction pathway in mammalian cells. We used Fura-2 (1μM) to measure calcium change in induced neural progenitor cells (iNPCs) and HEK293 cells. In the presence of a magnetic field of 100 mT, 40.5±3.5% of 200 iNPCs and 25±3.0 % of 200 HEK293 cells showed Fura-2 signal increases of 2.3±0.2 and 2.0±0.2 times, respectively. However, the calcium activation was 100% abolished in 200 iNPCs and HEK293 cells after treatment of 100 ng/ml pertussis toxin which inhibits the function of CB1 through the ADP-ribosylation of the Gαi and Gαo. Furthermore, GCaMP6m and EPG were co-transfected in iNPCs. Magnetic field of 100 mT evoked 2.5±0.5 fold increase calcium responses in 36.5% of 200 cells within 10 sec. Administration of the pertussis toxin (100 ng/ml) completely abolished the stimulation, while atropine (10 μM), an antagonist of the muscarinic acetylcholine receptor that is considered unrelated to CB1 pathway, did not affect calcium responses.So far, our results suggest that CB1, Gαi3 and Gαo may interact with EPG and the interactions can be enhanced under a magnetic field. It will be important to elucidate the exact mechanism by which EPG works through, in order to optimize and improve this magnetogenetics technology.

Disclosures: S. Xu: None. **Z.M. Kranz:** None. **X. Zhang:** None. **S. Mitra:** None. **G. Pelled:** None. **A.A. Gilad:** None.

Poster

254. Novel Approaches in Neuromodulation I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 254.08/CC1

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant R01NS098231
NIH Grant R01NS104306

Title: Behavioral response to magnetic fields in glass catfish has implications on neuromodulation technology

Authors: *R. HUNT[1], G. SALDANA DE JIMENEZ[1], A. GILAD[2,1,3], G. PELLED[2,1,3];
[1]Inst. for Quantatative Hlth. Sci. and Engin., [2]Biomed. Engin., [3]Dept. of Radiology, Michigan State Univ., East Lansing, MI

Abstract: *Kryptopterus bicirrhis* (Glass Catfish) has been known to elicit a behavioral response when exposed to magnetic fields. Recently, we have identified and cloned a new gene that we termed the electromagnetic perceptive gene (EPG) which when expressed in mammalian cells causes increases in intracellular calcium levels in response to magnetic stimulation (Krishnan et al. *Sci Rep*, 2018). It is of significance to understand the mechanisms by which the EPG functions, to investigate if there are additional genes associated with magnetic responses, and to develop additional magnetic sensing tools. Therefore, this study is designed to characterize the behavioral responses of *K. bicirrhis* to magnetic fields. Thirteen *K. bicirrhis* were placed in a radial y-maze and exposed to a Neodymium Rare Earth Magnet which produced a static magnetic field of 450 mT or a sham stimulus. Both magnet and sham stimulus were placed at the end arm one of the Y-maze, 10 cm from the end of the arm. Each of the three arms of the y-maze was 60 cm long, the central area had the dimensions 10x10x10 cm. Water conditions in the maze were kept constant and experiments were completed between 11am and 4pm to eliminate behavioral changes due to feeding and light cycle. Three starting positions were tested by barricading fish at the end of differing arms. Fish were placed in an arm that contained the magnet, or in a neighboring arm with no magnet. Each condition was repeated 4 times on different days for a total of 24 trials. Each trial was recorded by a high-resolution video camera that was positioned above the Y maze. Each trail lasted for 30 min. Recordings were analyzed by radial-maze tracking software written in Matlab according to Delcourt et al., *Behav Res Method,* 2018. This software identifies individual fish and track its movement with a 60 Hz resolution. The results show that fish consistently swam away from the arm with the magnet and spent the least time in it. When averaging the entire time course of 30 min, 1.17 ± 0.19 fish spent time in the arm with the magnet, compared to 5.167 ± 1.87 fish in neighboring arms. We found that the fish consistently swim away from the arm with the magnet (Student t-test $p<0.01$). We will build

upon results from this study to further characterize fish responses to different magnetic fields and frequencies, and test magnetic responses in an EPG knockout *K. bicirrhis.*

Disclosures: R. Hunt: None. **G. Saldana De Jimenez:** None. **A. Gilad:** None. **G. Pelled:** None.

Poster

254. Novel Approaches in Neuromodulation I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 254.09/CC2

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant R01NS098231
NIH Grant R01NS104306

Title: Developing hardware platforms for magnetogenetics in cells and systems

Authors: *R. ASHBAUGH[1,2], A. EFREMOV[1], L. UDPA[1], A. GILAD[3,2,4], G. PELLED[3,2,4]; [1]Electrical and Computer Engin., [2]Inst. of Quantitative Hlth. Sci. and Engin., [3]Biomed. Engin., [4]Dept. of Radiology, Michigan State Univ., East Lansing, MI

Abstract: New technologies to remotely control neuronal function using EPG (Electromagnetic-Perceptive-Gene)-based magnetogenetics (Krishnan et al., *Sci Reports*, 2018) and thermal-based magnetogenetics (Stanley, et al., *Nat Medicine*, 2015; Chen et al., *Science*, 2015) are growing in importance as a tool for non-invasive cellular stimulation. Many current solutions rely on permanent magnets or single coil electromagnets, however, these have multiple drawbacks. Firstly, placing a permanent magnet in close proximity to cells can be cumbersome, and secondly, it is difficult to control the magnitude and direction of applied magnetic stimulus, especially when studying multiple cells at a time.
Here we present novel electromagnet designs allowing hands free and reproducible delivery with customizable stimulation patterns of magnetic stimulus for a variety of targets. In particular, we present two approaches with improved capabilities; one that can be used for generating a localized focused magnetic field and a second based on Helmholtz coils that generate a uniform field over a larger area for targeting multiple cells.
Electrophysiology approaches are essential for investigating network and signal transduction pathways associated with magnetogenetics, as well as optimizing stimulation parameters. For slice patch-clamp and in-vivo electrophysiology recordings we designed two ferromagnetic core electromagnets. The first design used a conical core for the constrained setup of the patch-clamp electrophysiology recordings of rat brain slices and showed the capability to elicit consistent stimulation. The second design used a pencil-shaped core for in-vivo stimulation which was used to evoke multi-unit activity in neurons within the visual cortex of rats expressing EPG, showing

an increase of multi-unit activity. Magnetic stimulus produced by the designs are current dependent and reached as high as 15 mT with the conical core and 125 mT with the pencil core. We also designed an innovative Helmholtz coil based system to remotely stimulate cells and tissue cultures inside a fully enclosed apparatus such as a microscope or bioluminescence system. This design was used to stimulate cells in a 35 mm plate inside a fluorescence microscope and generated magnetic field strengths as high as 1.5 mT. This experimental setup has produced fluorescence in induced neuroprogenitor cells co-transfected with EPG and GCaMP. We are currently working on designing coils to uniformly stimulate 96 well plates to allow high throughput measurements. Our system shows promise to be an inexpensive and adaptable platform to deliver magnetic stimulus to a variety of biomedical applications.

Disclosures: R. Ashbaugh: None. **G. Pelled:** None. **A. Gilad:** None. **A. Efremov:** None. **L. Udpa:** None.

Poster

254. Novel Approaches in Neuromodulation I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 254.10/CC3

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant R01NS098231

Title: Electromagnetic perceptive gene (EPG) expression in the hippocampus reduces seizure activity in a kainic acid rat model of acute epilepsy

Authors: A. METTO[1,2], R. ASHBAUGH[2,3], G. PELLED[1,2,4];
[1]Biomed. Engin., [2]Inst. for Quantitative Hlth. Sci. and Engin., [3]Electrical and Computer Engin., [4]Dept. of Radiology, Michigan State Univ., East Lansing, MI

Abstract: Medial temporal lobe epilepsy (TLE) is the most prevalent form of epilepsy with focal onset seizures. Thirty percent of patients do not respond to anti-epileptic drugs, and are not good candidates for resective surgery. For these patients, neuromodulation strategies including deep brain stimulation and vagus nerve stimulation have shown success. However, these procedures are very complex and may pose serious risks to the patients.
We propose a novel, non-invasive gene-based intervention to alleviate seizure frequency and severity. Our laboratory has newly discovered a gene in the glass catfish, the electromagnetic perceptive gene (EPG) which responds to magnetic fields (Krishnan et al., *Sci Reports*, 2018). A seizure is characterized by abnormal hypersynchronous firing of neuronal populations. We hypothesize that the electric current produced by these neuronal networks induces a magnetic field that activates the EPG, which subsequently suppresses seizures or prevents them from further spreading, in a closed-loop fashion.

Our approach utilizes an acute kainic acid (KA) model of TLE in adult Wistar Furth rats. In the experimental group (n=4), EPG was stereotaxically injected in the CA3 region of the right hippocampus using a viral vector (AAV-CamKII-EPG-IRES-GFP). Control rats received either no injection or an injection of a control virus (AAV-CamKII-IRES-GFP) (n=5). Two to three weeks following stereotaxic procedure, intrahippocampal injection of KA (0.2 µg/0.2 µl) was administered to the same area of the right hippocampus. Tungsten microelectrodes were used to obtain hippocampal local field potential recordings (LFPs) and multi-unit activity (MUA) starting at 10 minutes after KA injection.
Seizure detection was performed using MATLAB. First, we used a band-pass filter and identified spiking activity. A seizure was defined by spikes that have an inter-spike interval greater than 0.05 s and less than 3 s, have more than 4 spikes in a train, and the train lasts at least for 7.5 s. A seizure episode that was at least 10 s apart from the next train was considered a distinct episode.
We found that the seizures in rats that expressed EPG were less frequent (6.75±3.3 vs. 11.6±6.0 in control), shorter in duration (33.73±14.9 s vs. 245.02± 423.8312 s in control) and had a delayed onset (14.12 mins vs. 12.27 mins in control).
These preliminary findings suggest that magnetogenetics via EPG may be an effective strategy to alleviate seizure severity in a non-invasive, closed loop and cell specific fashion.

Disclosures: **A. Metto:** None. **R. Ashbaugh:** None. **G. Pelled:** None.

Poster

254. Novel Approaches in Neuromodulation I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 254.11/CC4

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant R01NS098231
NIH Grant R01NS072171

Title: Non-invasive neuromodulation using tms and magnetogenetics alleviate pain associated with peripheral nerve injury in rats

Authors: *C. CYWIAK[1,2], A. METTO[2], M. ZHONG[2], G. PELLED[1,2,3];
[1]Biomed. Engin., [2]Inst. of Quantitative Hlth. Sci. and Engin., [3]Dept. of Radiology, Michigan State Univ., East Lansing, MI

Abstract: Twenty million Americans suffer from peripheral nerve injury (PNI). These patients often develop chronic pain and sensory dysfunctions. In the past decade, neuroimaging studies showed that these changes are associated with altered neural function and maladaptive plasticity in the somatosensory cortex (Li et al,. *PNAS,* 2011). We tested if neuromodulation techniques

would be effective in alleviating post-injury pain. Here we tested two non-invasive neuromodulation approaches: repetitive transcranial magnetic stimulation (rTMS) which is a non-invasive brain stimulation shown to induce long lasting neuronal excitability; And with the Electromagnetic-perceptive (EPG) which is a novel gene that we discovered and cloned from *Kryptopterrus bicirrhis* (Glass Catfish), and demonstrated that it responds to electromagnetic field stimulation (Krishnan et al, *Sci Reports*, 2018). We performed forepaw denervation on 29 rats, and a battery of behavioral testing to characterize sensorimotor and pain associate with the injury. The results show that rats that received TMS therapy every other day, for 30 days, starting the day after PNI (Acute, n=6) showed enhanced mobility in the beam walking test compared to rats that received rTMS therapy starting 3-weeks after injury (Delayed, n=6) and injured rats that received no therapy (No-rTMS, n=6)(Acute, 6.29±0.43s; Delayed, 7.05±0.36s; No-rTMS, 26.40±3.42s; $p<0.05$). They also showed decreased depression and anxiety in the open field test (Acute, 0.0473±0.0016m/s; Delayed, 0.038±0.0028m/s; No-rTMS, 0.0233±0.0014m/s; $p<0.05$), and in the novel object recognition test (Acute, 9.33 ±1.53; Delayed, 8±2.32 ; No-rTMS 1.5±1.504 n=6; $p<0.05$). The latter cognitive tests indicate that animals that are stimulated by this neuromodulation techniques suffer from less pain. In addition, fMRI results demonstrated enhanced neuroplasticity in the Acute group as was determined by the extent of activation (Acute, 123.4±22.95 pixels; No-rTMS, 73.6±22.06 pixels; $p<0.05$). We stereotactically injected the EPG to the somatosensory cortex contralateral to the injured limb (AAV-CamKII-EPG-GFP) a week after PNI procedure. We placed an electromagnet generating a field of ≈26mT, for 16 min, every day for 30 days. The results show that EPG stimulation led to decreased depression and anxiety in the open field test (EPG, 0.0405±0.0028m/s; n=6, No-EPG, 0.025±0.006m/s, n=4; $p<0.05$), and in the novel object recognition test (EPG, 7.16±1.178; n=6, No-EPG, 1.75±0.75, n=4; $p<0.05$). Together, these results suggest that rTMS and EPG treatment can be an effective approach for alleviating pain and facilitating rehabilitation in the weeks after PNI.

Disclosures: **C. Cywiak:** None. **G. Pelled:** None. **A. Metto:** None. **M. Zhong:** None.

Poster

254. Novel Approaches in Neuromodulation I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 254.12/CC5

Topic: I.08. Methods to Modulate Neural Activity

Support: NIGMS COBRE P20GM103645

Title: A novel model-informed TMS protocol to mimic the circuit effects of endogenous beta activity

Authors: *D. D. SLIVA[1], R. JAYARAM[2], J. OSTROWSKI[2], I. PENG[2], S. R. JONES[1];
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Abstract: Beta frequency activity (15-29Hz) is observed across multiple cortical regions, associated with inhibited sensory perception and motor action, and disrupted in a variety of neuropathology. Despite its prominence, whether and how beta exerts a causal influence on cortical function remains unknown. This knowledge gap limits the ability to reproduce the effects of beta using pharmacology or non-invasive brain stimulation in order to impact behavior and/or develop novel treatment methods. Several prior attempts to modulate behavior using non-invasive brain stimulation have utilized rhythmic stimulation protocols that attempt to mimic or enhance endogenous brain rhythms, including beta. However, we have shown that MEG-measured beta activity in primary somatosensory cortex (SI) is not purely "rhythmic", and can be more accurately characterized as an amalgamation of transient ~150ms "beta events" with a stereotyped waveform (Sherman et al 2016). The rate and timing of such events prior to a tactile stimulus drive an association between high beta power and decreased tactile detection (Shin et al 2018). Based on these findings, we propose that stimulation protocols targeting beta activity should take the features and transient dynamics of individual beta events into consideration. We have also developed a modeling framework designed to reproduce macroscale MEG/EEG activity based on known biophysics (HNN: http//hnn.brown.edu), and employed it to interpret neural circuit mechanisms underlying the generation of beta events (Sherman et al 2016). Motivated by this work, here we describe a novel model-informed transcranial magnetic stimulation (TMS) protocol designed to mimic the natural transient and intermittent expression of endogenous beta events. We hypothesize that applying this protocol over SI during a tactile detection task will inhibit detection in an analogous way as endogenous beta by invoking similar inhibitory circuit dynamics. We show that beta event waveforms are robust enough to be detected in sensor-level EEG data, thus allowing for identification and analysis of beta event features during concurrent TMS-EEG. We also provide evidence for rhythmic evoked potentials immediately following beta events. These "beta-evoked potentials" provide further mechanistic insight into functional after-effects of beta events, and serve as a marker for TMS protocol development. Finally, we demonstrate how our unique modeling framework can be used to develop and test hypotheses regarding potential similarities in circuit-level mechanisms underlying the effects of beta and TMS on neural dynamics and tactile perception.

Disclosures: D.D. Sliva: None. **R. Jayaram:** None. **J. Ostrowski:** None. **I. Peng:** None. **S.R. Jones:** None.

Poster

254. Novel Approaches in Neuromodulation I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 254.13/CC6

Topic: I.08. Methods to Modulate Neural Activity

Title: Motor cortex excitability is not reliably modulated by transcranial static magnetic field stimulation

Authors: *T. KAMMER, B. ALEX, S. LORENZ;
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Abstract: Recently, modulatory effects of a static magnetic field stimulation (tSMS) on the excitability of the motor cortex have been reported. In our previous study (Kufner et al. 2017) we failed to replicate these results. It was suggested that the lack of modulatory effects was due to the use of an auditory oddball task in our study (Foffani et al. 2017).
In the present study, we aimed to evaluate the role of an auditory oddball task on the effects of static magnetic field stimulation on motor cortex excitability. Therefore, in a within-subject-design we directly compared stimulation with and without oddball task.
In 18 subjects the left motor cortex was exposed to a permanent magnet over the scalp for 10 minutes each. In one of the two sessions subjects had to perform an auditory oddball task during the exposure to the magnet, whereas there was no task during exposure in the other session. Motor cortex excitability was measured before and after tSMS by means of motor evoked potentials (MEPs).
No modulation of motor cortex excitability was observed in any condition. However, when data were pooled regarding the order of the sessions, a trend for an increase of excitability was observed in the first session compared to the second session.
Our results confirm our previous study, i.e. that the suppressive effect of 10 min tSMS can't be directly replicated. Furthermore, the auditory oddball task seems to be neutral with respect to MEP modulation. Fluctuations in the amplitudes of MEPs may possibly mask weak modulatory effects but may also lead to false positive results if the number of subjects in a study is too low. In addition, there might be a habituation effect to the whole procedure.

Disclosures: **T. Kammer:** None. **B. Alex:** None. **S. Lorenz:** None.

Poster

254. Novel Approaches in Neuromodulation I

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Program #/Poster #: 254.14/CC7

Topic: I.08. Methods to Modulate Neural Activity

Support: MOST 106-2410-H-010-004-MY2
MOST 108-2420-H-010-001

Title: Dynamic modulation of excitation and inhibition equilibrium by high frequency repetitive transcranial magnetic stimulation in the early visual cortex

Authors: *S. N. LIN, Y. LIEN, C. LIN, L. CHANG;
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Abstract: Visual cortex excitability has been widely studied by occipital transcranial magnetic stimulation (TMS). Previous studies suggested the plasticity-like effect after repetitive TMS (rTMS) had resulted from the TMS-induced alterations in neurochemistry and synaptic function. Although current models between animal and human brain studies came to a consensus that both excitation and inhibition neural systems modulate the plasticity across the lifetime and tasks, the underlying mechanism of the neurochemistry function, the cortical reaction of rTMS-mediated neural plasticity and rTMS-based therapies remained incompletely comprehended. To address the questions, we applied either high frequency (HF, 10Hz) or sham stimulation over visual cortex on healthy volunteers, and observed the phosphene thresholds (PT), magnetic resonance spectroscopy(MRS)-derived glutamate/glutamine (Glx) and γ-aminobutyric acid (GABA) before, 0.5hr after, 3.5 hr after, and 24 hr after rTMS intervention. Then, the excitation/inhibition ratio (E/I ratio) was calculated by the concentration of Glx over GABA. Surprisingly, we found that the cortical sensitivity to single TMS pulse increased in the early stage (within 0.5hr) (baseline vs. 0.5hr, $p<0.05$) and recovered few hours later; however, the shifted neurochemical balance (E/I ratio) in local tissue seems to be affected longer (+3.5hr) (baseline vs. 3.5hr, $p<0.01$; 0.5hr vs. 3.5hr, $p<0.01$) than PT measurements in the HF group. On the other hand, changes in GABA concentration were more prominent than those in glutamate, and the dynamical changes of the E/I balance were mostly driven by the inhibitory neurotransmitter, GABA. Thus, our results showed that 10 Hz rTMS might shift E/I balance in neural networks toward more excitation and instability state. The negatively correlated feature between Glx and PT was also altered at 3.5h, indicating temporarily decoupled cortical excitatory factors. Here, we propose that short-term HF rTMS intervention may facilitate an extended time window toward increasing cortical excitability through weakened inhibition that possibly allow more plasticity induced.

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Poster

254. Novel Approaches in Neuromodulation I

Location: Hall A

Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 254.15/CC8

Topic: I.08. Methods to Modulate Neural Activity

Support: CIHR 154292

Title: The effect of high-frequency rTMS on inhibitory neural circuit interactions in the motor cortex

Authors: *N. M. DRUMMOND[1], W. HOEFSLOOT[2], C. GUNRAJ[1], R. CHEN[1];
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Abstract: High frequency repetitive transcranial magnetic stimulation (10 Hz - rTMS) over the motor cortex has been repeatedly explored as a treatment for chronic pain patients with positive outcomes. Multiple theories have been put forth regarding the mechanism behind rTMS induced pain reduction, but to date the exact mechanism is still unclear. One theory highlights the importance of inhibitory neural circuits in the motor cortex. Although research has been carried out on the effect of rTMS on these neural circuits individually with paired-pulse TMS, no study investigated the effect of rTMS on the interactions of these inhibitory neural circuits. This can be done with a triple-pulse TMS technique. The purpose of this experiment was to explore how excitatory rTMS (intermittent 10 Hz) of the motor cortex effects short-interval intracortical inhibition (SICI), long-interval intracortical inhibition (LICI) and their interactions. It was hypothesized that rTMS would induce disinhibition in both SICI and LICI alone, as well as disinhibition of SICI in the presence of LICI. Participants completed two visits separated by at least a week, receiving either real rTMS or sham rTMS in a randomized cross-over design with pre- and post-TMS measurements. Inhibitory circuit measurements were made at baseline and at 10, 30 and 50 minutes after receiving rTMS delivered in a posterior-anterior direction in accordance with pain treatment protocols. The results revealed that despite an increase in motor cortical excitability following rTMS, there were no significant differences in the inhibitory circuits between real and sham rTMS. These findings suggest that the mechanism of analgesia in chronic pain patients following rTMS is not due to the modulation of intracortical motor circuits. Further testing needs to be done to expand our knowledge of the underlying mechanisms of rTMS induced analgesia.

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Poster

254. Novel Approaches in Neuromodulation I

Location: Hall A

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Program #/Poster #: 254.16/CC9

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH-MH112142

Title: Modulation of decision-making behavior by deep brain focused ultrasound stimulation in nonhuman primates

Authors: *F. A. MUNOZ[1], A. MEANEY[1], K. LIU[2], A. GROSS[2], A. POULIOPOULOS[3], E. KONOFAGOU[2], V. P. FERRERA[4];
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Abstract: Noninvasive Focused Ultrasound Stimulation (FUS) has emerged as new approach for modulating brain function and behavior. Cognitive effects of FUS in neuromodulation have been little explored in awake primates. In this study, we applied FUS to dorsal striatum (putamen) during the performance of a decision making task, using a single transducer and neuronavigation system, and evaluated the effect on decision accuracy and reaction time (RT). Two Rhesus monkeys were trained using a touchpanel display. A visual cue was presented on one side of the display and the monkey reached out and touched the cue to initiate a trial. The cue was either a vertical or horizontal bar that indicated the reward size (5 or 1 drop) for trial. After touching the cue, two patches of visual motion appeared simultaneously, one with random motion and the other with some degree of coherent motion; the monkey was rewarded for touching the coherent motion patch. The strength of motion modulated the difficulty of the decision. Data collection was done in 2 conditions: sessions without (n=31) and with sonication (n=88). Sonication was applied to one hemisphere while monkeys performed the task. A pair of function generators produced 10 ms duration pulses of a 500 kHz sine wave with a duty cycle of 2 Hz for 120 s after 200 trials. The FUS pressure was 200 or 400 kPa. The analysis estimated the detection threshold between conditions using a generalized linear model (GLM) to determine the dependence of accuracy and reaction time on side of stimulus presentation, motion strength, hemisphere sonicated, reward size, and sonication pressure (0, 200, 400 kPa). The accuracy threshold for all sonication pressures was in the range of 0.36+0.03 in one subject and 0.25+0.02 for the second, showing that the FUS had a non-significant effect in accuracy; whereas the GLM showed that the strength of motion, side of presentation, and reward size were significant predictors ($P<0.05$). In contrast, RT was reduced depending on the intensity of the stimulation ($P<0.05$) in both subjects. The effect was contralateral to the hemisphere that was stimulated. In conclusion, we present a clear modulation of behavior in realtime by FUS stimulation to dorsal striatum during a decision making task.

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Poster

254. Novel Approaches in Neuromodulation I

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Time: Sunday, October 20, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 254.17/CC10

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant MH111763

Title: Ovine model assessment of sonication parameters on transcranial focused ultrasound-mediated neuromodulation

Authors: K. YOON[1], W. LEE[2], J. LEE[2], L. XU[2], ***S.-S. YOO**[2];
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Abstract: Focused ultrasound (FUS)-mediated neuromodulation, with advantages of exquisite spatial selectivity and depth penetration, has drawn attention as a potential non-invasive brain stimulation modality for various neurologic and psychiatric applications. We examined the effects of FUS sonication parameters on transient modification of excitability of somatosensory area (of the hind leg) and its thalamic projection using an ovine model, with an aim to translate this technique to potential clinical practice. As guided by anatomical and functional neuroimaging data specific to each animal, 250 kHz FUS was transcranially applied to the relevant brain areas in sheep (n = 10) across multiple sessions to examine the effects of various combinations in sonication parameters. The degree of excitation and suppression of the sonicated brain areas was assessed through electrophysiological responses. We found that the modulatory effects were transient and reversible while a certain range of pulsed sonication parameters outperformed on eliciting or suppressing regional brain activity, which indicates the presence of optimal sonication parameter for the modulatory effects to take place. Magnetic resonance imaging and histological analysis conducted at different time points after the last sonication session, as well as behavioral observations, indicate that repeated exposure to FUS does not damage the underlying brain tissues. Our results suggest that FUS-mediated non-invasive, region-specific bimodal neuromodulation can be safely achieved in an ovine model, indicating its potential for translation into human studies.

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Poster

254. Novel Approaches in Neuromodulation I

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Program #/Poster #: 254.18/CC11

Topic: I.08. Methods to Modulate Neural Activity

Support: NSF grant 1707865

Title: Focused ultrasound transiently increases membrane conductance in isolated crayfish axon

Authors: *J.-W. LIN[1], **F. YU**[1], W. S. MÜLLER[2], G. EHNHOLM[3], Y. OKADA[2];
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Abstract: Ultrasound (US) has been demonstrated to be an effective neuromodulatory modality. US can propagate through the skull and be focused deep into brain with high spatial resolution. Thus it has the potential for non-invasive transcranial stimulation in humans. Despite demonstrated effects of US on neural tissues, the mechanisms underlying this modulation remain unclear at single cell level. We use motor axons of the crayfish opener preparation to examine the effects of US on excitable cells. US tone bursts (2.1 MHz for 5 ms, 1-mm focal diameter, 0.1-1 MPa) consistently generated a fast hyperpolarization and, more importantly, stochastically triggered depolarization or a train of action potentials. The depolarization persisted in the presence of blockers for the main voltage-gated channels in this preparation (1 µM TTX (I_{Na}), 50 µM ZD7288 (I_h), and 200 µM 4-aminopyridine (I_K)), suggesting that voltage gated channels are unlikely to underlie the depolarization, although a possible role for mechanoreceptors has not been ruled out. Electrophysiological parameters of the depolarization were measured in 11 preparations. The depolarization typically started during the tone burst, with a mean latency, measured from the beginning of the US burst, of 3.35 ± 0.53 ms, and its duration measured at 25% of peak amplitude was highly variable, ranging from 20ms to 200 s with an average of 2.13 ± 0.87 s. Depolarization amplitude averaged 10.1 ± 2.09 mV and could be as large as 60 mV. There was a decrease in membrane resistance during the depolarization, indicating the presence of a US induced conductance (g_{us}). To calculate the conductance and reversal potential (E_{us}) of g_{us}, we measured the membrane potential and resistance during US-triggered depolarization. The estimated E_{us} was -8.4 ± 2.3 mV (n=5), suggesting g_{us} is likely to be non-selective for ions. The value of g_{us} ranged 10-100 times larger than the leak conductance. Modeling studies from other groups have suggested that pressure waves from an US tone burst may perturb the lateral motion of phospholipids, creating nanopores in the membrane. The nanopores, if ion-permeable, could be the basis of g_{us}. This study has characterized membrane conductance triggered by US burst and provided a high resolution description of events during US induced neuromodulation.

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Poster

254. Novel Approaches in Neuromodulation I

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Topic: I.08. Methods to Modulate Neural Activity

Support: KHIDI Grant HI14C3477
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Title: Noninvasive spinal cord stimulation by low-intensity ultrasound

Authors: E. KIM[1], J. KUM[1,2], ***H. KIM**[1,2];
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Abstract: Stimulation of the spinal cord is used to alleviate chronic pain and manage motor-related neurologic disorders. The typical approach is to deliver a mild electric current precisely at a targeted spinal segment. Despite the practical benefits, the method requires permanent fixation of the electrodes in epidural space and periodic surgical intervention to replace the system power supply. Recently, low-intensity ultrasound has been utilized to modulate neural circuit with high precision in a noninvasive fashion. Previous studies have shown the ability of ultrasound to excite and suppress brain network and stimulate the abducens and sciatic nerves, but not on the spinal cord. In this work, we investigate the ability of low-intensity ultrasound as a noninvasive tool to stimulate the spinal cord. A portable, custom-made transducer with a fundamental frequency of 450 kHz was attached on mouse paraspinal muscle above the intact T13 spinal vertebra. Acoustic stimulation was delivered to the L3 segment and verified by measuring electromyography (EMG) from the associated hind limb through induced motor responses. The stimulation parameters (1 kHz of pulse-repetition frequency, 0.5 ms of tone-burst duration, and 300 ms of sonication duration) were selected as excitatory protocol from our preliminary trials and based on other published resources. The ultrasound-based spinal cord stimulation was able to elicit the robust twitch of hind limbs with a delay of approximately 23 ms from the stimulation onset, while identical acoustic stimulation targeting muscle did not show any motor responses. The results verified the ability of low-intensity ultrasound to stimulate neurons at the spinal cord. Ultrasonic spinal cord stimulation as a promising application of therapeutic ultrasound may provide new hope for patients with chronic pain, locomotion deficit, and other disorders of the spinal cord.

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Poster

254. Novel Approaches in Neuromodulation I

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Program #/Poster #: 254.20/CC13

Topic: I.08. Methods to Modulate Neural Activity

Support: R01 NS098781A1

Title: Ultrasound suppression of seizures in 4-aminopyridine model of epilepsy

Authors: *B. ELAHIAN[1], C. SMITH[1], P. O'BRIEN[2], R. ARAVALLI[2], D. DARROW[3], E. EBBINI[2], T. NETOFF[1];
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Abstract: *Introduction*:Reversible neuromodulation with ultrasound (US) stimulation has been more than 60 years ago (Fry.F.J,et al,1958). US has been considered as a potential non-invasive therapy for many neurological disorders. A few studies have shown the effects of US stimulation on seizure activity in a focal acute seizure model. However, its effects on generalized seizures has not been well demonstrated. In this study we test seizure suppression using a Low Intensity Focused Ultrasound (LIFU) applied with a dual-mode mutli-transducer ultrasound array (DMUA) applied to the ventral thalamus in a generalized seizure rat model induced by intraperitoneal injection of 4-aminopyridine. *Method:*10 Sprague-Dawley rats were used (all male, 280-350 gr, Charles River Laboratories Minneapolis, MN) in accordance to a UMN IACUC approved protocol. The animals were divided into two groups: 4AP + US (N=5), 4AP (N=5). To deliver ultrasound, a 64-element DMUA prototype (Haritonova et al, 2015), was used to generate modulated tFUS patterns to the ventral thalamus. The seizure model was induced by 4AP injection (2 mL of 4−AP solution at 2 mg/mL in saline) intraperitoneally (IP). Ultrasound was applied periodically for 5 minutes on and 5 minutes off. Surgical anesthesia for all rats was induced using 5-10 mg/kg Xylazine, 40-90 mg/kg Ketamine.For recording, four burr holes were drilled using a dental drill for placement of epidural electrodes. Four 200-μm-diameter stainless steel with 0.5-mm tip electrodes were implanted bilaterally epidurally on cortex at 2 mm and 5 mm posterior and +/- 2 mm lateral from bregma).*Results*:Across 5 rats we recorded 19 seizures. With application of ultrasound the first seizure was observed in all animals before 2700 seconds following 4AP injection. Of the 19 seizures observed, only 2 seizure onsets occurred during ultrasound. Epileptiform activity developed over time and spiking activity was seen within 20 minutes and seizures within 30- 40 minutes. When 4-AP was injected IP and no ultrasound was applied, seizure onsets occurred later. *Conclusion:*We hypothesize that the blood brain barrier (BBB) opening caused by US may promote ictogenesis by allowing 4-AP to cross into the brain. The application of ultrasound promoted onset of seizures and spikes rates in LFP ipsilateral to US therapy compared to contralateral. This application suppress the seizure onset. A rebound in seizures immediately after termination of ultrasound therapy was observed.

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